Veterinary and Comparative Oncology

WILEY

Veterinary and Comparative Oncology

ORIGINAL ARTICLE

SHINE – Validation of Near Infrared Fluorescence Lymphography Against Lymphoscintigraphy for Sentinel Lymph Node Biopsy in Dogs With Mast Cell Tumours

L. E. Chiti[1] | P. Beer[1] | S. M. Ohlerth[2] | S. Hartnack[3] | M. C. Nolff[1]

[1]Clinic for Small Animal Surgery, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland | [2]Clinic of Diagnostic Imagining, Vetsuisse Factulty, University of Zurich, Zurich, Switzerland | [3]Section of Veterinary Epidemiology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Correspondence: L. E. Chiti (lavinia.chiti@gmail.com)

Received: 28 September 2024 | **Revised:** 28 February 2025 | **Accepted:** 30 March 2025

Funding: This work was supported by UZH Postdoc Grant (FK-23-073), Albert-Heim-Stiftung für Kleintiere (ASH158), Stiftung für Kleintiere.

Keywords: dog | fluorescent-guided surgery | lymphoscintigraphy | mast cell tumour | near-infrared | sentinel lymph node

ABSTRACT

Lymphoscintigraphy is the gold standard among sentinel lymph node (SLN) mapping techniques. Unfortunately, lymphoscintigraphy is not readily accessible, leading to the need for validation of alternative techniques. The aim of this study is to compare near-infrared fluorescence lymphography (NIRF-L) with lymphoscintigraphy for SLN resection in MCT and assess the impact of intraoperative NIRF guidance. Forty-eight dogs with 60 MCT were included in this prospective, blinded, randomised controlled trial. Dogs underwent preoperative lymphoscintigraphy and were then randomised into two groups: in the treatment group ($n = 30$) intraoperative NIRF-L was performed; in the control group ($n = 30$) no intraoperative guidance was implemented. Detection rate, concordance, sensitivity, and negative predictive values were recorded for NIRF-L and lymphoscintigraphy. Surgical time and length of surgical incision were compared between treatment and control groups with the Wilcoxon test (5% significance). Detection rate was 100% for NIRF-L and 98% for lymphoscintigraphy. Discordance occurred in one case. Sensitivity of NIRF-L was 93.7% (95% C.I. 74.3%–99.3%) and negative predictive value ranged between 91.1% and 98.6% with a prevalence of nodal metastases of 61% and 18%. Based on the overlapping of the confidence intervals, NIRF-L was not statistically different to lymphoscintigraphy for sensitivity. Lymphadenectomy was unsuccessful in 4/30 (13%) cases in the control group. Surgical time and incision were significantly shorter in the treatment group ($p < 0.001$; $p = 0.001$). Based on our results, NIRF-L is a valid alternative to lymphoscintigraphy for SLN removal in MCT. Moreover, it improves the success of lymphadenectomy, reduces surgical time, and incision length compared to an unguided technique.

1 | Introduction

Canine integumentary mast cell tumours (MCT) spread to the lymph nodes as the first metastatic site, and the presence of nodal metastases has been correlated with reduced survival times [1–5]. Lymphadenectomy is thus recommended in the surgical management of MCT, given its importance for correct staging, treatment recommendations, and the positive effect on survival times [2, 5–7]. However, surgeons must face two main difficulties when performing lymphadenectomies: (1) The tumour's draining lymph node – the sentinel lymph node (SLN), differs from the clinically expected regional lymph node (RLN) in up to 28%–63% of dogs [8–11]. (2) The intraoperative identification of the SLN can be difficult if the nodes are clinically normal, leading to failure of unguided lymphadenectomy in up to 30% of cases [12–14].

[Correction added on 25 April 2025, after first online publication. Both funding and ethics statements were corrected.]

Various mapping techniques have been described to guide the identification and removal of the SLN pre- and intra-operatively in dogs with MCT. Lymphoscintigraphy is considered the gold standard because, based on the available evidence, it allows for successful SLN removal in the vast majority of cases, with detection rates as high as 91%–100% [8, 15, 16]. This assumption is also corroborated by data reported for human malignancies such as cutaneous melanoma, squamous cell carcinoma, and breast cancer, over a thirty-year period [17, 18]. However, the availability of lymphoscintigraphy in veterinary practice is limited due to the relatively high costs and restrictions on storage and handling of the radioactive tracers.

More accessible SLN mapping techniques have been described for clinical use in dogs with MCT and include indirect radiographic (IRL) [19–21] or computed tomographic lymphography (ICTL) [9, 11, 22, 23], contrast-enhanced ultrasound (CEUS) [10], and indocyanine green (ICG)-based near-infrared fluorescent lymphography (NIRF-L) [11, 12]. Indirect CT lymphography has become increasingly popular for SLN mapping in dogs with various malignancies. While early clinical studies reported highly variable SLN detection rates, ranging from 42% to 90% [9, 22, 24–26], two more recent studies have shown detection rates as high as 97% for oral tumours and MCT [27, 28]. The main drawbacks of IRL/ICTL are the involvement of repeated doses of ionising radiation and the lack of intraoperative guidance, which may increase the risk of missing SLNs during surgical dissection if an intraoperative mapping technique is not combined [11, 22, 26]. Similar to IRL/ICTL, CEUS does not allow for intraoperative localization of the SLN [29]. For instance, Fourier et al. (2020) reported a preoperative SLN detection rate of 95.5% with CEUS for canine MCT, but in 10/62 cases the SLN could not be found intraoperatively [10]. The main advantage of NIRF-L is that, due to a penetration depth of the NIR light of 10–12 mm, the lymphatic drainage pathways towards the SLN can be visualised transcutaneously in real-time, thus providing both lymphatic mapping and intraoperative guidance for lymphadenectomy [30, 31]. In dogs with MCT, detection rates of 80%–95% have been reported for NIRF-L. [11, 12] In a recent study, the technique allowed for the identification of metastatic SLNs in 68%, while only 33% metastatic nodes were identified when unguided lymphadenectomy was performed [12].

Despite these encouraging data, NIRF-L has not been compared to the current gold standard – lymphoscintigraphy, for SLN removal in dogs with MCT. This step is crucial to validate the technique and support its implementation in veterinary practice. Hence, the primary aim of this study was to assess the diagnostic performances (detection rate, sensitivity, negative predictive values) of ICG-based NIRF-L for SLN identification and removal in dogs with MCT and to compare it with the current gold standard, lymphoscintigraphy. As a secondary aim, the success rate of lymphadenectomy, surgical time, and length of surgical incision were compared between dogs undergoing unguided lymphadenectomy and those receiving intraoperative NIRF-L to guide SLN removal.

We hypothesized that detection rates of NIRF-L are similar to lymphoscintigraphy, that the two techniques would show concordance on the location of the SLN in at least 80% of cases, and that implementation of intraoperative NIRF-L would increase the success rate of lymphadenectomy and reduce the surgical time and length of incision compared to unguided lymphadenectomy.

2 | Materials and Methods

This prospective, blinded, randomised controlled trial was conducted in a single institution from February 2022 to April 2024. We included consecutive client-owned dogs with cytologically/histologically confirmed integumentary MCT, amenable to curative-intent surgical excision (2 cm lateral margins and one deep fascial plane OR proportional margins) [32–34] and lymphadenectomy. Dogs that received neoadjuvant chemotherapy (excluding preoperative corticosteroids or antihistaminic) or radiotherapy, that had previous surgical procedures within the tributary lymphosomes, and that were presented with stage IV disease were excluded. Multiple MCTs were included only if they were sufficiently distant from each other to prevent potential overlapping lymphatic drainage or lymphosomes. The study was approved by the national authorities under the registration number ZH183/2021 and it was pre-registered at www.animalstudyregistry.org (doi: 10.17590/asr.0000314). Owners had to sign a written informed consent for participation in the study.

The study design is summarised in the flow-chart (Figure 1). All included dogs underwent preoperative lymphoscintigraphy under deep sedation prior to surgery, as previously described [8, 16]. Nano-sized human serum albumin labelled with technetium-99 metastable was injected intradermally in four quadrants peritumorally and repeated static planar images (120 s/frame, matrix size 256 × 256) were acquired with a gamma-camera (Equine Scanner HR – SCINTRON) constantly until the first draining node was identified. Lymphoscintigraphy was performed and interpreted by one of the surgeons (L.E.C), who completed a PhD on this topic and has 6 years of clinical experience with the technique, in collaboration with a board-certified radiologist (S.O.) of our institution's radiology department. The SLN was identified as an area of radioactive uptake directly connected with the primary tumour via a radioactive tract (lymphatic drainage). Only SLNs with a direct connection to the primary tumour were classified as first draining, or tier-1, nodes. Nodes that received indirect drainage through a tier-1 SLN were designated as tier-2 nodes. The median lymphoscintigraphy time was 20 min (range 10–107 min).

Lymphadenectomy and MCT removal were scheduled within 15 days of lymphoscintigraphy. Surgical procedures were performed by a board-certified surgeon (M.C.N.) who was blinded to the results of preoperative lymphoscintigraphy. Covariate adaptive randomization was used to assign included dogs to two groups of intraoperative lymph node management: (a) Treatment Group = dogs undergoing NIRF-L guided SLN removal; (b) Control Group = dogs undergoing unguided RLN/SLN removal. This randomization strategy was chosen to avoid a potential source of bias from the imbalance of lymphocenter location between groups [35].

Dogs in the treatment group received an intradermal injection of 0.25–1 mL (median: 0.45 mL) ICG solution (2.5 mg/mL) intradermally in four quadrants around the tumour in the case of

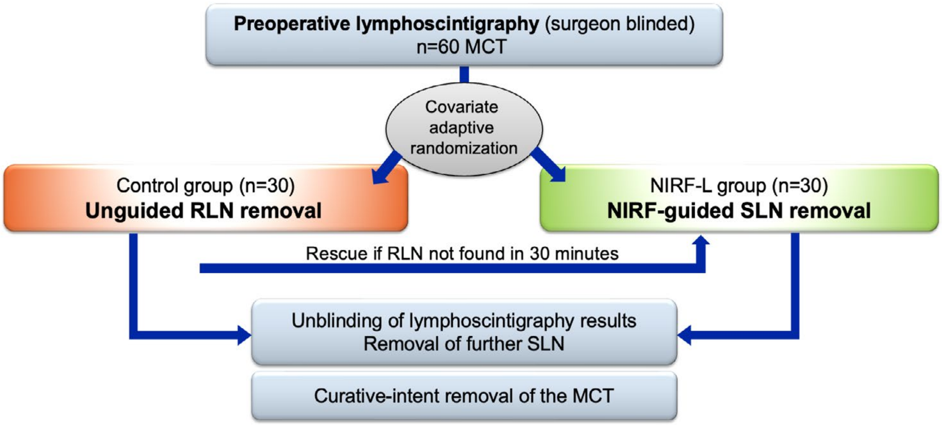

FIGURE 1 | Flow-chart of the study.

cutaneous MCT and subcutaneously in the case of subcutaneous MCT, and a dedicated NIR camera system (Visionsense Iridium) was used to detect the fluorescent signal intraoperatively. After transcutaneous visualisation of the lymphatics and identification of the SLN, the incision was centred above the signal corresponding to the anticipated node. If intraabdominal location of the SLN was suspected, a caudal median or lateral celiotomy was performed. The fluorescent signal then guided the surgeon during deeper tissue dissection until the SLN was identified and removed. Complications related to tracer administration were recorded.

Dogs in the control group received unguided RLN extirpation based on the lymphosomes described by Suami et al. [36].

After completion of NIRF-L guided or unguided lymphadenectomy, the results of lymphoscintigraphy were unblinded, and if further SLN had been identified with lymphoscintigraphy, they were also removed. The primary MCT was then excised with 2 cm lateral margins and one deep fascial plane, if the location and size of the tumour allowed, or with the widest margins possible otherwise.

In both groups, lymphadenectomy was deemed unsuccessful if the surgeon was not able to find the SLN or RLN within 30 min. In this case, due to ethical reasons, dogs of the control group received "rescue" NIRF-L to aid nodal extirpation. Time from skin incision at the lymphadenectomy site to identification and removal of the RLN or SLN, and total time for lymphadenectomy (from skin incision to skin closure) were recorded. The length of the surgical incision at the lymphadenectomy site was also recorded.

The excised nodes and MCT were submitted for histopathology. The nodes were graded according to Weishaar et al. (2014) [2]. Cutaneous MCT were graded according to Patnaik [37] and Kiupel [38], and completeness of excision was evaluated with a cross-section technique.

Detection rate and concordance between NIRF-L and lymphoscintigraphy were calculated after unblinding. Detection rate was defined for each technique as a proportion, which is the number of procedures that allowed for the identification of at least one SLN divided by the total number of procedures performed. Concordance was defined as total if the two techniques identified the same SLN, partial if one of the two techniques identified further SLN, and no concordance if the two techniques identified different SLN.

To evaluate the potential benefits of NIRF-L over unguided lymphadenectomy, the success rate (proportion of cases in which the node was successfully removed) time for lymphadenectomy, and length of surgical incision were compared between the treatment and control groups. The correspondence between RLN and SLN identified with NIRF-L and lymphoscintigraphy was also assessed.

Included dogs were rechecked at 10 days after surgery, and postoperative complications of lymphadenectomy or tumour excision were recorded. Follow-up rechecks were planned at 3, 6 and 12 months to assess potential tumour progression.

2.1 | Statistical Analysis

The sample size was calculated to obtain a power of 80% for the comparison of the success of lymphadenectomy between the two groups, considering a probability of success of 95% in the NIRF-L group and 67% in the control group, based on previously published data [12].

Numerical data were summarised with descriptive statistical measures. A two-samples Wilcoxon test was used to compare the distribution of continuous variables between the groups, and Fisher's exact test was used for categorical data. Sensitivity and negative predictive value (NPV) were calculated to assess the diagnostic performances of lymphoscintigraphy and NIRF-L. For these calculations, test results were defined as follows: true positive if SLN were removed and at least one was metastatic at histopathology; true negative if SLN were removed but were nonmetastatic at histopathology; false negative if SLN were removed but metastases occurred in another node during the follow-up time. False positives would occur if SLN are removed, result metastatic at histopathology but they are ultimately nonmetastatic; this definition does not apply in this case because a node is defined metastatic if metastases are detected at histopathology (gold standard). For this reason, specificity, likelihood ratios and positive predictive value were not reported as specificity could only be 100%. To calculate NPV, a prevalence of nodal metastases of 18%–61% was considered [5, 11]. A 95% binomial confidence interval (95% C.I.) was obtained using Jeffreys approach. For these calculations, HN2 and HN3 nodes were considered metastatic whereas HN0 and HN1 were considered non metastatic [2].

Overlapping of the 95% C.I. for the accuracy measures of the two mapping techniques was then assessed to determine whether NIRF-L did not significantly differ from the gold standard lymphoscintigraphy.

Median follow-up time was calculated with the reverse Kaplan-Meier method. Significance was set to $p = 0.05$. The analyses were performed by R statistical software version 4.2.1 (R Core Team (2024). R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>).

3 | Results

Forty-eight dogs with 60 MCT were included and equally assigned to the treatment and control groups. Dogs' and tumours' characteristics, including MCT histopathology, are summarised in Table 1. These variables did not differ statistically between the groups (Table 2).

In 7 cases lymphoscintigraphy and surgery were performed on the same day; in all other cases, surgery was not performed on the same day to reduce exposure of the personnel to radiations (1–13 days apart, median 3 days apart).

3.1 | Comparison of NIRF-L and Lymphoscintigraphy

Lymphoscintigraphy identified an SLN in 59/60 cases (detection rate: 98.3%), and NIRF-L identified an SLN in 30/30 cases of the treatment group (detection rate: 100%). Lymphoscintigraphy failed for an MCT located on the right proximal neck; in this case, NIRF-L identified an ipsilateral superficial cervical node. In 7 cases in the treatment group (23.3%), fluorescent signal from the lymph node was not visible before skin incision ($n = 3$ superficial cervical nodes; $n = 4$ medial iliac nodes) (Figure 2). However, in the three cases of superficial cervical nodes, the signal from the lymphatics was visible and guided the dissection of the superficial planes until visualisation of the node, whereas for the four cases of medial iliac nodes, an intracavitary location was suspected based on Suami's lymphosomes, and since no other superficial nodes had fluorescent signal, a celiotomy was performed and the signal of the node became visible. In the remaining 23 cases (76.7%), a fluorescent signal was clearly visible through the skin before incision (Figure 3).

Lymphoscintigraphy and NIRF-L showed partial or total concordance in all but one case (96.7%) for which lymphoscintigraphy failed. Total concordance was recorded in 23 cases (76.7%) (Figure 4). Partial concordance occurred in 7 cases (23.3%), in which NIRF-L identified further SLNs.

TABLE 1 | Distribution of dogs' and tumours' variables in the study population.

Variable continuous	Mean	Median	Standard deviation
Age (years)	7.302	6.35	2.67
Weight (kg)	24.61	13	18.98
MCT size (mm)	18.8	13	16.51
Categorical			**Number (%)**
Breed			
French bulldog			6 (12.5%)
Mix breed			9 (16.67%)
Retriever			14 (29.17%)
Other breeds (< 3 each)			20 (41.67%)
Sex			
Female intact			2 (4.17%)
Male castrated			14 (29.16%)
Male intact			16 (33.33%)
Female castrated			16 (33.33%)
Type			
Cutaneous			45 (75%)
Subcutaneous			15 (25%)
Presentation			
First presentation			57 (95%)
Recurrence			15 (25%)
Ulceration			
No			9 (15%)
Yes			3 (5%)
Patnaik grade			
I			6 (10%)
II			35 (58.33%)
II			15 (25%)
NA (subcutaneous, scars)			15 (25%)
Kiupel grade			
Low			37 (61.67%)
High			8 (13.33%)
NA (subcutaneous, scar)			15 (25%)
Surgical margins			
Tumour-free			56 (93.3%)
Infiltrated			4 (6.67%)
Highest HN[a]			
HN0			20 (33.33%)
HN1			9 (15%)
HN2			25 (41.67%)
HN3			6 (10%)

Abbreviation: NA, Not assessed.
[a][2] Weishaar et al. 2014.

TABLE 2 | Comparison of significant variables between the control and the study group.

Continuous variables	Median, control group	Median, study group	Wilcoxon rank
Incision length (mm)	70	47.5	$W = 1167.6$; $p = 0.0004$*
Time until nodal removal (min)	7	5	$W = 1347.5$; $p = 0.0098$*
Time until wound closure (min)	14	11.5	$W = 1073.5$; $p = 0.0495$*
Node size (mm)	15	13	$W = 1865$ $p = 0.1689$
Age (years)	6.35	6.50	$W = 285$ $p = 0.9586$
Weight (kg)	23.6	26	$W = 257.5$ $p = 0.536$
MCT size (mm)	17	11.5	$W = 443.5$ $p = 0.2497$
Categorical variables	**Distribution, control group (number)**	**Distribution, study group (number)**	**Fisher's exact test**
Breed	French bulldog (5) Mix breed (2) Other < 3 (12) Retriever (5)	French bulldog (1) Mix breed (6) Other < 3 (8) Retriever (9)	$p = 0.1003$
Sex	Male intact (8) Male castrated (5) Female intact (1) Female castrated (10)	Male intact (8) Male castrated (9) Female intact (1) Female castrated (6)	$p = 0.5778$
Type	Cutaneous (23) Subcutaneous (7)	Cutaneous (22) Subcutaneous (8)	$p = 1$
Presentation	First presentation (29) Recurrence (1)	First presentation (28) Recurrence (2)	$p = 1$
Ulceration	Yes (6) No (24)	Yes (3) No (27)	$p = 0.4716$
Patnaik grade	I (2) II (18) III (3) NA (7)	I (4) II (17) III (1) NA (8)	$p = 0.6752$
Kiupel grade	Low (17) High (6) NA (7)	Low (20) High (2) NA (8)	$p = 0.4217$

Abbreviation: NA, Not assessed.
*Significant *p*-value.

Sensitivity for NIRF-L was 93.7% (95% C.I. 74.3%–99.3%). Negative predictive value ranged between 91.1% and 98.6% when considering a prevalence of nodal metastases of 61% and 18%, respectively.

For lymphoscintigraphy, sensitivity was 91.2% (95% C.I. 78.3%–97.4%) and NPV varied between 87.9% and 98.1% when considering a prevalence of nodal metastases of 61% and 18%. Based on the overlapping of the 95% C.I., sensitivity did not differ statistically between NIRF-L and lymphoscintigraphy.

3.2 | Comparison of Guided and Unguided Lymphadenectomy

In the control group, unguided lymphadenectomy failed in 4 cases (13.3%), but rescue NIRF-L ultimately allowed for removal of the SLN; in one case, the fluorescent signal was disturbed by extravasation of ICG due to previous dissection, but the procedure was still successful.

A total of 126 nodes were removed, 65 in the treatment group and 61 in the control group. Multiple SLNs were removed in

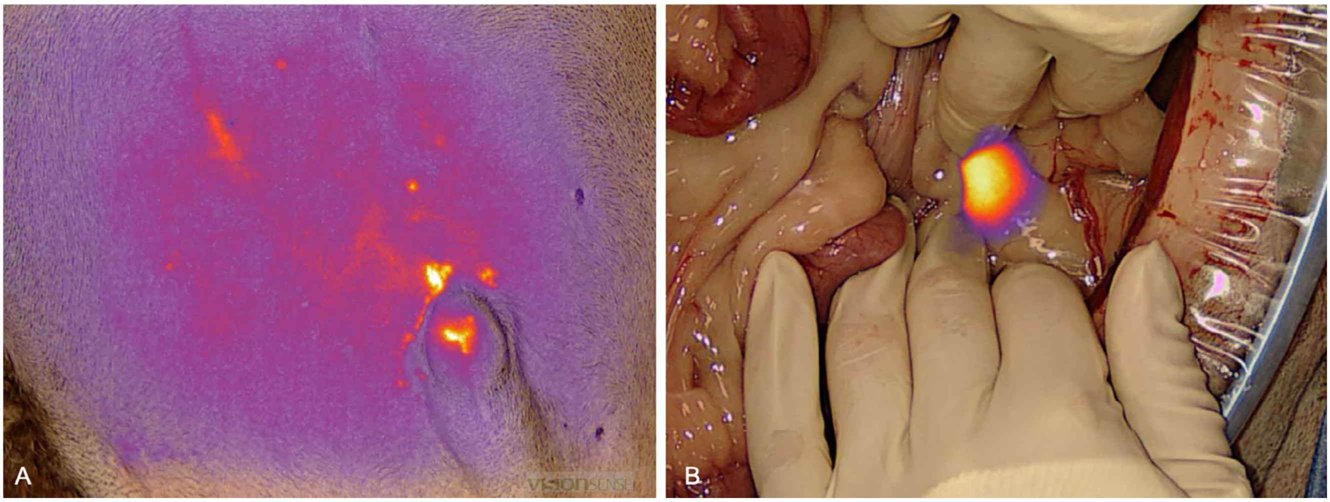

FIGURE 2 | Absence of transcutaneous signal in a case of a medial iliac sentinel lymph node (A) and clear signal after celiotomy to the medial iliac lymph node (B).

FIGURE 3 | Transcutaneous lymphography in various anatomical locations: (A) axillary lymph node (B) inguinal lymph node (C) superficial cervical lymph node.

19/30 (63.3%) cases in the treatment group and 16/30 (53.3%) in the control group, and they belonged to different lymphocenters in 12 (40%) and 11 (36.7%) cases, respectively. The median size of the excised nodes was 15 mm in the control group and 13 mm in the treatment group, and the difference was not statistically significant ($p = 0.1689$). The median lymphadenectomy incision length was 70 mm in the control group and 47.5 mm in the treatment group, and the difference was statistically significant ($p = 0.0004$). The time for node removal and until closure of the lymphadenectomy wound were 7 min and 14 min in the control group, and 5 min and 11.5 min in the treatment group; these measures also differed statistically ($p = 0.0098$; $p = 0.0495$) (Table 2).

The SLN identified with NIRF-L or lymphoscintigraphy differed from the RLN in 24 cases (40%). At histopathology, 61 (48.4%) nodes were HN0, 21 (16.7%) HN1, 36 (28.6%) HN2, and 8 (6.3%) HN3. In 7 cases (11.6%), implementation of a mapping technique led to an upstaging, and in 2 cases, stage remained unchanged but an additional HN2 node was removed. In two cases of partial concordance between lymphoscintigraphy and NIRF-L, the latter allowed for excision of an additional HN2 node.

3.3 | Complications and Follow-Up

In one dog, MCT degranulation occurred after peritumoral injection of ICG, but this caused only local swelling and did not have systemic effects; no other complications related to tracer administration were recorded. Postoperative complications at the tumour site occurred in 14 (23.3%) cases (only 1/14 cases required surgical revision). Postoperative complications at the lymphadenectomy site occurred in 16 (26.7%) cases (8 in the treatment group and 8 in the control group); the most common were lymphoedema and seroma (11/16) and required a surgical revision in 3/16 cases.

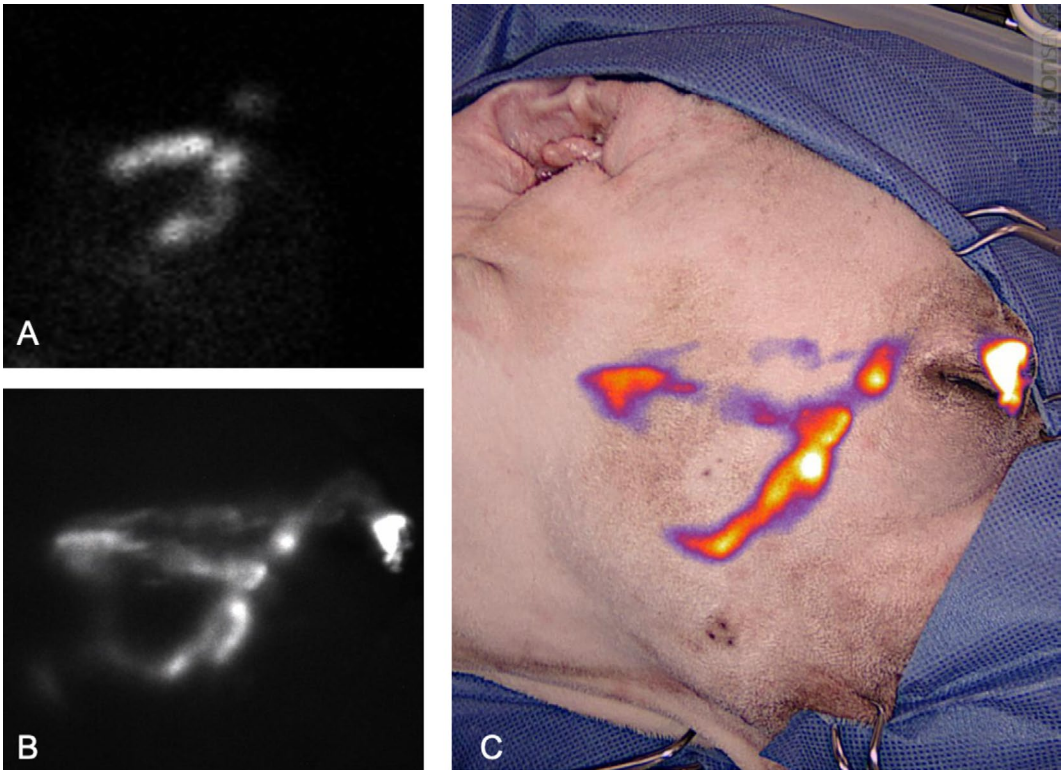

FIGURE 4 | Mast cell tumour of the upper eyelid in a dog. Correspondence of drainage pathways to the mandibular and parotid nodes in lymphoscintigraphy (A) and NIRF-L (B): Fluorescence image, (C): Overlaid image.

During the follow-up time (median: 474 days; 95% CI: 339–542), three dogs developed local recurrence with nodal and distant metastases, one dog developed concurrent nodal and distal metastases, and nine dogs developed de-novo MCT. At the end of the study, five dogs have died due to tumour-related causes (median follow-up: 502 days) and one (follow-up: 10 days) due to causes unrelated to the study and MCT.

4 | Discussion

Based on the results of the present study, we could accept the hypothesis that NIRF-L is as reliable as lymphoscintigraphy for SLN removal in dogs with MCT. In the study population, NIRF-L allowed for identification of at least one SLN in all cases, leading to a detection rate of 100%, which compares favourably with the 98% detection rate that we recorded for lymphoscintigraphy. Failure of lymphoscintigraphy occurred in a MCT located on the neck, for which the ipsilateral superficial cervical node was then identified as the SLN with NIRF-L. In this case, the failure of lymphoscintigraphy was probably due to the close proximity of the SLN to the injection site, which caused an overlapping of the radiation flare of the primary tumour with the hotspot of the SLN and impeded the visualisation of the latter. This phenomenon, usually referred to as "shine through", constitutes one of the main limitations of lymphoscintigraphy in people, and it has promoted the implementation of dual tracers consisting of a combination of a radioactive nano colloid and ICG [39, 40]. In a prospective investigation on 30 people with oral squamous cell carcinoma undergoing SLN removal guided by a dual tracer, 12% of SLN would have been missed without ICG due to their location close to the primary tumour [41]. Although comparable data are lacking in veterinary oncology, this observation may suggest a particular value of NIRF-L when the SLN is located close to the injection site.

The 100% detection rate that we report for NIRF-L is higher than what has been reported for dogs with MCT in two previous investigations [11, 12]. This discrepancy may be due to the use of imaging systems with variable diagnostic capabilities as well as to a learning curve. The imaging system that we used in this study outperformed other systems for open fluorescent imaging in previous studies, and this may have positively influenced our detection rate [42, 43]. Furthermore, although evidence is lacking on the impact of experience with NIRF-L on the nodal detection rate in dogs, at our institution we noticed an increase in the SLN detection rate from 83% to 100% in the past 5 years, suggesting that the experience of the surgeon may also play a role in the success of the procedure.

Significant concordance between lymphoscintigraphy and NIRF-L occurred in the sample population: NIRF-L and lymphoscintigraphy identified the same SLN in 96.7% of cases, although in 23.3% NIRF-L guided the intraoperative dissection of further SLN, belonging to different lymphocenters, that were not identified preoperatively with lymphoscintigraphy. Similarly to our study, NIRF-L has been reported to allow for identification of a higher number of SLN than lymphoscintigraphy in women with endometrial and breast cancer [44–46]. In two dogs of our study population, an HN2 SLN would have been missed by lymphoscintigraphy and was identified only by NIRF-L. In both cases, the removal of these extra SLN did not influence the staging, as the SLN identified by lymphoscintigraphy were also HN2. However, due to the therapeutic impact of the removal of

HN2 nodes in canine MCT, identification and removal of these nodes may still be clinically relevant [6, 47].

With 93.7% sensitivity, SLN removal guided by NIRF-L and followed by histopathology should be considered a highly accurate diagnostic test to confirm the presence of nodal metastases in dogs with MCT. Furthermore, the high negative predictive value suggests that this modality can reliably exclude the presence of nodal metastases. It should be, however, underscored that, to estimate the measurements of diagnostic accuracy, the "test" was considered the mapping technique followed by histopathology; both NIRF-L and lymphoscintigraphy, however, are pure mapping techniques and should be therefore regarded as methods to identify and visualise the SLN while they do not directly give information on the metastatic status of the SLN, which must be assessed with histopathology on the removed nodes.

Based on the overlapping of the 95% C.I., NIRF-L was equal to lymphoscintigraphy with regard to sensitivity, while specificity was equal to 100% for both techniques due to the study design. This result confirms that NIRF-L is a valid alternative to lymphoscintigraphy for dogs with MCT, and it is in line with evidence in humans with cutaneous melanoma, for which a recent meta-analysis reported no overall difference in the identification of metastatic patients or false negative rate between lymphoscintigraphy and NIRF-L [48].

In 23.3% of dogs, NIRF signal was not visible before skin incision, but was clearly identified immediately after dissection of the superficial planes. In four cases, the SLN was an intraabdominal node, and therefore the limited depth of penetration of the NIR light did not allow for transcutaneous lymphography. The other three cases were dogs > 30 kg with high body condition scores, and the SLN was a superficial cervical node. Although this lymphocenter should be normally superficially located, in these specific cases the superficial cervical node was presumably more deeply located due to the size of the dogs and deposition of adipose tissue in this region, thus hampering transcutaneous visualisation of NIR signal. Based on these results, it seems reasonable to argue that anticipation of drainage to a deeply located lymphocenter may be an indication to combine NIRF-L to a preoperative mapping technique. Stoffels and colleagues (2015) reported a similar experience in a cohort of 80 people with skin melanoma that underwent SLN removal guided by NIRF-L and lymphoscintigraphy: despite an intraoperative detection rate of 96% for NIRF-L, the fluorescent signal was not visible before skin incision in 21% of patients, leading the authors to suggest a combination of the two techniques among patients for whom location of the SLN cannot be predicted [49].

The secondary aim of the present study was to assess the benefits of intraoperative NIRF-L guidance compared to unguided lymphadenectomy. In our cohort of dogs, unguided RLN removal failed in 13% of cases, in which rescue NIRF-L ultimately allowed for successful completion of the procedure. Conversely, lymphadenectomy was successfully completed in all dogs that received intraoperative NIRF-L. This result underscores the importance of the implementation of an intraoperative mapping technique to improve the success rate of lymphadenectomy and is consistent with the results of previous studies. In a recent study on dogs with MCT, the nodal detection rate increased from 74% without intraoperative guidance to 83% with the implementation on intraoperative NIRF-L. [12] Likewise, in dogs with oral tumours, the implementation of intraoperative NIRF-L raised the detection rate to 100% compared to 42% when preoperative ICTL was not combined with an intraoperative mapping technique [26]. Incorporation of other intraoperative mapping techniques, such as methylene blue or anchor wires, has also been reported to significantly raise the nodal detection rate in dogs and cats with various malignancies [13, 14].

In the study population, incision length and surgical time for lymphadenectomy were significantly shorter when lymphadenectomy was intraoperatively guided by NIRF-L compared to the unguided technique. Indeed, visualisation of the fluorescent signal facilitates the identification and dissection of the SLN and could therefore allow the surgeon to respect the SLN while minimising the surgical dose. The incision length and surgical time were chosen as measures of the surgical dose in the present study as they can be objectively quantified and are less subjected to sources of bias. However, even if the study was conducted blindly, the fact that the surgeon could or could not see the fluorescent signal may have been a source of bias, especially for the incision length. Therefore, to better assess the benefits of intraoperative fluorescent guidance, the incidence and severeness of surgical complications should also be compared between guided and unguided lymphadenectomy. In the study population, postoperative complications related to lymphadenectomy occurred in 16 (26.7%) cases and were mostly self-limiting, in accordance with previously reported data [50]. Although the relatively low number of recorded complications did not allow for statistical comparison of their incidence between the two groups (unguided vs. NIRF-L guided lymphadenectomy), they occurred in an equal number of dogs in each group. Based on our results, it is therefore not possible to argue that implementation of NIRF-L reduces the incidence of postoperative complications compared to unguided lymphadenectomy.

Finally, the SLN identified with NIRF-L or lymphoscintigraphy did not correspond to the clinically expected RLN in 40% of cases, and in 12% of cases, nodal metastases would have been missed with the removal of the RLN only. The fact that nodal metastases can be misdiagnosed in a significant proportion of dogs with MCT without the implementation of a SLN mapping technique has been well documented and represents the main argument to support the implementation of SLN mapping and removal as standard of care for dogs with MCT [8, 11, 26].

The present study features a prospective, randomised and blinded design and a sample size calculation, which indeed limited the possible sources of bias for the comparison of the diagnostic performances of NIRF-L and lymphoscintigraphy and for the assessment of its benefits compared to unguided lymphadenectomy. However, the sample size and limited median follow-up time (474 days) did not allow for investigating the influence of NIRF-L guided SLN removal on the long-term oncological outcome of dogs with MCT. Future studies are warranted to establish if the implementation of specific SLN mapping techniques with various detection rates and diagnostic performances has an impact on the oncological outcome of dogs with MCT.

In conclusion, NIRF-L is as reliable as the gold-standard lymphoscintigraphy for SLN identification and removal in dogs, and NIRF-L guided SLN removal followed by histopathology can accurately identify and rule out the presence of nodal metastases. Implementation of NIRF-L compared to unguided lymphadenectomy allows for a higher success rate, reduced surgical times, and shorter surgical incisions. These results should encourage the implementation of NIRF-L for optimal management of dogs with MCT.

Ethics Statement

The study was conducted on client-owned live dogs with naturally occurring cancers. Owner signed a written consent to the procedures, and the study was ethically approved at our institution with the TVA number ZH183/202.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

References

1. C. T. Hume, M. Kiupel, L. Rigatti, F. S. Shofer, K. A. Skorupski, and K. U. Sorenmo, "Outcomes of Dogs With Grade 3 Mast Cell Tumors: 43 Cases (1997-2007)," *Journal of the American Animal Hospital Association* 47, no. 1 (2011): 37–44.

2. K. M. Weishaar, D. H. Thamm, D. R. Worley, and D. A. Kamstock, "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," *Journal of Comparative Pathology* 151, no. 4 (2014): 329–338.

3. C. Chalfon, R. Finotello, S. Sabattini, et al., "Patterns of Nodal Metastases, Biological Behaviour and Prognosis of Canine Mast Cell Tumours of the Pinna: A Multi-Institutional Retrospective Study," *Veterinary and Comparative Oncology* 21, no. 2 (2023): 332–338.

4. R. Ferrari, L. Marconato, P. Buracco, et al., "The Impact of Extirpation of Non-palpable/Normal-Sized Regional Lymph Nodes on Staging of Canine Cutaneous Mast Cell Tumours: A Multicentric Retrospective Study," *Veterinary and Comparative Oncology* 16, no. 4 (2018): 505–510.

5. H. Baginski, G. Davis, and R. P. Bastian, "The Prognostic Value of Lymph Node Metastasis With Grade 2 MCTs in Dogs: 55 Cases (2001–2010)," *Journal of the American Animal Hospital Association* 50, no. 2 (2014): 89–95.

6. L. Marconato, G. Polton, D. Stefanello, et al., "Therapeutic Impact of Regional Lymphadenectomy in Canine Stage II Cutaneous Mast Cell Tumours," *Veterinary and Comparative Oncology* 16, no. 4 (2018): 580–589.

7. C. Chalfon, S. Sabattini, R. Finotello, et al., "Lymphadenectomy Improves Outcome in Dogs With Resected Kiupel High-Grade Cutaneous Mast Cell Tumours and Overtly Metastatic Regional Lymph Nodes," *Journal of Small Animal Practice* 63, no. 9 (2022): 661–669.

8. R. Ferrari, L. E. Chiti, M. Manfredi, et al., "Biopsy of Sentinel Lymph Nodes After Injection of Methylene Blue and Lymphoscintigraphic Guidance in 30 Dogs With Mast Cell Tumors," *Veterinary Surgery* 49, no. 6 (2020): 1099–1108, https://doi.org/10.1111/vsu.13483.

9. J. Lapsley, G. M. Hayes, V. Janvier, et al., "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," *Veterinary Surgery* 50, no. 1 (2021): 133–141.

10. Q. Fournier, F. Thierry, M. Longo, et al., "Contrast-Enhanced Ultrasound for Sentinel Lymph Node Mapping in the Routine Staging of Canine Mast Cell Tumours: A Feasibility Study," *Veterinary and Comparative Oncology* 19, no. 3 (2021): 451–462.

11. A. Alvarez-Sanchez, K. L. Townsend, L. Newsom, M. Milovancev, E. Gorman, and D. S. Russell, "Comparison of Indirect Computed Tomographic Lymphography and Near-Infrared Fluorescence Sentinel Lymph Node Mapping for Integumentary Canine Mast Cell Tumors," *Veterinary Surgery* 52, no. 3 (2023): 416–427.

12. P. Beer, C. Rohrer-Bley, and M. C. Nolff, "Near-Infrared Fluorescent Image-Guided Lymph Node Dissection Compared With Locoregional Lymphadenectomies in Dogs With Mast Cell Tumours," *Journal of Small Animal Practice* 63, no. 9 (2022): 670–678.

13. M. Rossanese, A. Pierini, G. Pisani, et al., "Ultrasound-Guided Placement of an Anchor Wire or Injection of Methylene Blue to Aid in the Intraoperative Localization and Excision of Peripheral Lymph Nodes in Dogs and Cats," *Journal of the American Veterinary Medical Association* 260, no. S1 (2022): S75–S82.

14. M. C. C. De Souza, M. C. Flecher, F. M. Arrais, B. V. de Sena, A. Giuliano, and R. d. S. Horta, "Comparison of Surgical Resection of Axillary Lymph Nodes in Dogs With Mammary Gland Tumors With or Without Sentinel Lymph Node Visualization With Patent Blue Dye," *Frontiers in Veterinary Science* 10 (2023): 1149315, https://doi.org/10.3389/fvets.2023.1149315.

15. D. R. Worley, "Incorporation of Sentinel Lymph Node Mapping in Dogs With Mast Cell Tumours: 20 Consecutive Procedures," *Veterinary and Comparative Oncology* 12, no. 3 (2014): 215–226.

16. M. Manfredi, D. De Zani, L. E. Chiti, et al., "Preoperative Planar Lymphoscintigraphy Allows for Sentinel Lymph Node Detection in 51 Dogs Improving Staging Accuracy: Feasibility and Pitfalls," *Veterinary Radiology & Ultrasound* 62, no. 5 (2021): 602–609.

17. I. J. den Toom, K. Boeve, D. Lobeek, et al., "Elective Neck Dissection or Sentinel Lymph Node Biopsy in Early Stage Oral Cavity Cancer Patients: The Dutch Experience," *Cancers (Basel)* 12, no. 7 (2020): 1783.

18. M. G. Niebling, R. G. Pleijhuis, E. Bastiaannet, A. H. Brouwers, G. M. van Dam, and H. J. Hoekstra, "A Systematic Review and Meta-Analyses of Sentinel Lymph Node Identification in Breast Cancer and Melanoma, A Plea for Tracer Mapping," *European Journal of Surgical Oncology* 42, no. 4 (2016): 466–473, https://doi.org/10.1016/j.ejso.2015.12.007.

19. H. N. Brissot and E. G. Edery, "Use of Indirect Lymphography to Identify Sentinel Lymph Node in Dogs: A Pilot Study in 30 Tumours," *Veterinary and Comparative Oncology* 15, no. 3 (2017): 740–753.

20. A. De Bonis, F. Collivignarelli, A. Paolini, et al., "Sentinel Lymph Node Mapping With Indirect Lymphangiography for Canine Mast Cell Tumour," *Vet Sci* 9, no. 9 (2022): 484.

21. M. Annoni, S. Borgonovo, and M. Aralla, "Sentinel Lymph Node Mapping in Canine Mast Cell Tumours Using a Preoperative Radiographic Indirect Lymphography: Technique Description and Results in 138 Cases," *Veterinary and Comparative Oncology* 21, no. 3 (2023): 469–481.

22. F. Rossi, M. Körner, J. Suárez, et al., "Computed Tomographic-Lymphography as a Complementary Technique for Lymph Node Staging in Dogs With Malignant Tumors of Various Sites," *Veterinary Radiology & Ultrasound* 59, no. 2 (2018): 155–162.

23. J. A. Grimes, S. A. Secrest, M. L. Wallace, T. Laver, and C. W. Schmiedt, "Use of Indirect Computed Tomography Lymphangiography to Determine Metastatic Status of Sentinel Lymph Nodes in Dogs With a Pre-Operative Diagnosis of Melanoma or Mast Cell Tumour," *Veterinary and Comparative Oncology* 18, no. 4 (2020): 818–824.

24. J. A. Grimes, S. A. Secrest, N. C. Northrup, C. F. Saba, and C. W. Schmiedt, "Indirect Computed Tomography Lymphangiography With Aqueous Contrast for Evaluation of Sentinel Lymph Nodes in Dogs With Tumors of the Head," *Veterinary Radiology & Ultrasound* 58, no. 5 (2017): 559–564.

25. E. K. Randall, M. D. Jones, S. L. Kraft, and D. R. Worley, "The Development of an Indirect Computed Tomography Lymphography Protocol for Sentinel Lymph Node Detection in Head and Neck Cancer and Comparison to Other Sentinel Lymph Node Mapping Techniques," *Veterinary and Comparative Oncology* 18, no. 4 (2020): 634–644.

26. J. Wan, M. L. Oblak, A. Ram, A. Singh, and S. Nykamp, "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," *Veterinary and Comparative Oncology* 19, no. 2 (2021): 295–303.

27. B. Gianni, R. Franchi, M. Mattolini, et al., "CT Features of Cutaneous and Subcutaneous Canine Mast Cell Tumors and Utility of Conventional and Indirect Lymphography to Detect Clinically Unknown Mast Cell Tumors and to Map the Sentinel Lymph Nodes," *Veterinary Radiology & Ultrasound* 65, no. 2 (2024): 170–180.

28. S. Goldschmidt, N. Stewart, C. Ober, et al., "Contrast-Enhanced and Indirect Computed Tomography Lymphangiography Accurately Identifies the Cervical Lymphocenter at Risk for Metastasis in Pet Dogs With Spontaneously Occurring Oral Neoplasia," *PLoS One* 18 (2023): e0282500.

29. P. Beer, A. Pozzi, C. Rohrer Bley, N. Bacon, N. S. Pfammatter, and C. Venzin, "The Role of Sentinel Lymph Node Mapping in Small Animal Veterinary Medicine: A Comparison With Current Approaches in Human Medicine," *Veterinary and Comparative Oncology* 16, no. 2 (2018): 178–187.

30. K. L. Townsend, M. Milovancev, and S. Bracha, "Feasibility of Near-Infrared Fluorescence Imaging for Sentinel Lymph Node Evaluation of the Oral Cavity in Healthy Dogs," *American Journal of Veterinary Research* 79, no. 9 (2018): 995–1000.

31. C. Hirche, D. Murawa, Z. Mohr, S. Kneif, and M. Hünerbein, "ICG Fluorescence-Guided Sentinel Node Biopsy for Axillary Nodal Staging in Breast Cancer," *Breast Cancer Research and Treatment* 121, no. 2 (2010): 373–378.

32. K. Pratschke, M. Atherton, J. Sillito, and C. Lamm, "Evaluation of a Modified Proportional Margins Approach for Surgical Resection of Mast Cell Tumors in Dogs: 40 Cases (2008–2012)," *Journal of the American Veterinary Medical Association* 243 (2013): 1436–1441.

33. H. Saunders, M. J. Thomson, K. O'Connell, J. P. Bridges, and L. Chau, "Evaluation of a Modified Proportional Margin Approach for Complete Surgical Excision of Canine Cutaneous Mast Cell Tumours and Its Association With Clinical Outcome," *Veterinary and Comparative Oncology* 19, no. 4 (2021): 604–615.

34. T. Itoh, A. Kojimoto, K. Uchida, J. K. Chambers, and H. Shii, "Long-Term Postsurgical Outcomes of Mast Cell Tumors Resected With a Margin Proportional to the Tumor Diameter in 23 Dogs," *Journal of Veterinary Medical Science* 83, no. 2 (2021): 230–233.

35. K. Suresh, "An Overview of Randomization Techniques: An Unbiased Assessment of Outcome in Clinical Research," *J Hum Reprod Sci* 4, no. 1 (2011): 8.

36. H. Suami, S. Yamashita, M. A. Soto-Miranda, and D. W. Chang, "Lymphatic Territories (Lymphosomes) in a Canine: An Animal Model for Investigation of Postoperative Lymphatic Alterations," *PLoS One* 8, no. 7 (2013): e69222.

37. A. K. Patnaik, W. J. Ehler, and E. G. Macewen, "Canine Cutaneous Mast Cell Tumor: Morphologic Grading and Survival Time in 83 Dogs," *Veterinary Pathology* 21, no. 5 (1984): 469–474.

38. M. Kiupel, J. D. Webster, K. L. Bailey, et al., "Proposal of a 2-Tier Histologic Grading System for Canine Cutaneous Mast Cell Tumors to More Accurately Predict Biological Behavior," *Veterinary Pathology* 48, no. 1 (2011): 147–155.

39. R. de Bree, R. P. Takes, J. P. Shah, et al., "Elective Neck Dissection in Oral Squamous Cell Carcinoma: Past, Present and Future," *Oral Oncology* 90 (2019): 87–93.

40. I. J. Den Toom, K. Boeve, D. Lobeek, et al., "Elective Neck Dissection or Sentinel Lymph Node Biopsy in Early Stage Oral Cavity Cancer Patients: The Dutch Experience," *Cancers (Basel)* 12, no. 7 (2020): 1–13.

41. A. Christensen, K. Juhl, B. Charabi, et al., "Feasibility of Real-Time Near-Infrared Fluorescence Tracer Imaging in Sentinel Node Biopsy for Oral Cavity Cancer Patients," *Annals of Surgical Oncology* 23, no. 2 (2016): 565–572.

42. A. V. D Souza, H. Lin, E. R. Henderson, K. S. Samkoe, and B. W. Pogue, "Review of Fluorescence Guided Surgery Systems: Identification of Key Performance Capabilities Beyond Indocyanine Green Imaging," *Journal of Biomedical Optics* 21, no. 8 (2016): 80901.

43. L. E. Chiti, B. Husi, B. Park, et al., "Performance of Two Clinical Fluorescence Imaging Systems With Different Targeted and Non-Targeted Near-Infrared Fluorophores: A Cadaveric Explorative Study," *Frontiers in Veterinary Science* 10 (2023): 1091842, https://doi.org/10.3389/fvets.2023.1091842.

44. S. Togami, T. Ushiwaka, M. Fukuda, et al., "Comparison of Radio-Isotope Method With 99m Technetium and Near-Infrared Fluorescent Imaging With Indocyanine Green for Sentinel Lymph Node Detection in Endometrial Cancer," *Japanese Journal of Clinical Oncology* 52, no. 1 (2022): 24–28.

45. T. Sugie, T. Kinoshita, N. Masuda, et al., "Evaluation of the Clinical Utility of the ICG Fluorescence Method Compared With the Radioisotope Method for Sentinel Lymph Node Biopsy in Breast Cancer," *Annals of Surgical Oncology* 23, no. 1 (2016): 44–50.

46. A. Papadia, M. L. Gasparri, A. Buda, and M. D. Mueller, "Sentinel Lymph Node Mapping in Endometrial Cancer: Comparison of Fluorescence Dye With Traditional Radiocolloid and Blue," *Journal of Cancer Research and Clinical Oncology* 143, no. 10 (2017): 2039–2048.

47. L. Marconato, D. Stefanello, M. Kiupel, et al., "Adjuvant Medical Therapy Provides no Therapeutic Benefit in the Treatment of Dogs With Low-Grade Mast Cell Tumours and Early Nodal Metastasis Undergoing Surgery," *Veterinary and Comparative Oncology* 18, no. 3 (2020): 409–415.

48. M. Wölffer, R. Liechti, M. Constantinescu, I. Lese, and C. Zubler, "Sentinel Lymph Node Detection in Cutaneous Melanoma Using Indocyanine Green-Based Near-Infrared Fluorescence Imaging: A Systematic Review and Meta-Analysis," *Cancers (Basel)* 16, no. 14 (2024): 2523.

49. I. Stoffels, J. Dissemond, T. Pöppel, D. Schadendorf, and J. Klode, "Intraoperative Fluorescence Imaging for Sentinel Lymph Node Detection: Prospective Clinical Trial to Compare the Usefulness of Indocyanine Green vs Technetium Tc 99m for Identification of Sentinel Lymph Nodes," *JAMA Surgery* 150, no. 7 (2015): 617–623.

50. L. E. Chiti, E. M. Gariboldi, R. Ferrari, et al., "Surgical Complications Following Sentinel Lymph Node Biopsy Guided by γ-Probe and Methylene Blue in 113 Tumour-Bearing Dogs," *Veterinary and Comparative Oncology* 21, no. 1 (2023): 62–72.