

CASE REPORT

Intraoperative sentinel lymph node mapping with indocyanine green via video-assisted thoracoscopic surgery for primary pulmonary neoplasia in a dog

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Abstract

Objective: To report the use of intraoperative sentinel lymph node (SLN) mapping with indocyanine green (ICG) and near-infrared (NIR) imaging in a dog with a primary pulmonary carcinoma that underwent lung lobectomy via a video-assisted thoracoscopic approach.

Study design: Case report.

Animals: A 9-year-old female spayed Labrador retriever.

Methods: A solitary, 6.5 cm diameter, right caudal pulmonary mass was identified on contrast-enhanced thoracic and abdominal computed tomography, with no overt metastatic disease or other primary neoplastic diseases. The dog underwent video-assisted thoracoscopic surgery. A right-sided thoracoscopic approach was initially performed for right caudal pulmonary ligament transection and image-guided peritumoral (intrapulmonary) ICG injection. Due to the large size of the mass, a wound retractor was then placed in the right 6th intercostal space for right caudal lung lobectomy. Sentinel lymph node mapping was performed via NIR imaging, and the SLN was extirpated for histologic assessment.

Results: The right tracheobronchial lymph node was identified as sentinel via NIR fluorescence following peritumoral ICG injection. Right caudal lung lobectomy and right tracheobronchial lymph node extirpation were performed without complication, and histologic evaluation revealed a grade 1 pulmonary adenocarcinoma with pneumonia and a reactive lymph node.

Conclusion: Peritumoral ICG administration with NIR imaging appears to be an effective method for intraoperative SLN mapping in dogs with primary pulmonary tumors. The technique utilized in this report can be applied to open or minimally invasive procedures. Large-scale studies with SLN mapping are needed to determine an accurate incidence of nodal metastatic disease and any

Abbreviations: CT, computed tomography; ICG, indocyanine green; NIR, near-infrared; SLN, sentinel lymph node; VATS, video-assisted thoracoscopic surgery.

effect of extirpation of early metastatic disease via SLN mapping techniques on oncologic outcomes in dogs with primary pulmonary carcinoma.

1 | INTRODUCTION

Primary pulmonary tumors in dogs are generally malignant (carcinomas) with a high propensity for metastasis. The locoregional lymph nodes are a common site of metastasis, and nodal metastatic disease is associated with prognosis.^{1–4} Surgical biopsy of regional lymph nodes at the time of pulmonary tumor resection has therefore been recommended in dogs.^{5,6} However, data are limited regarding which lymph node(s) should be biopsied to assess for metastatic disease at the time of surgery. Determining if nodal metastasis has occurred, and in which lymph node, is challenging in dogs with primary pulmonary carcinoma for the following reasons: (1) variation in regional lymph node anatomy and lymph node drainage pattern from the lungs; (2) limitations in determining nodal metastasis on computed tomography (CT) with the potential for normal-appearing lymph nodes to be metastatic on histologic evaluation; and (3) difficulty in identifying tracheobronchial, pulmonary, and mediastinal lymph nodes intraoperatively due to their small size and locations near critical vascular structures.^{4,7,8} These findings highlight a need for standardization of lymph node staging in dogs with pulmonary tumors, with reliable techniques to identify and extirpate the first draining lymph node (i.e., the sentinel lymph node) for histologic evaluation in dogs with potentially occult metastatic lesions.

Sentinel lymph node (SLN) mapping has been effective for multiple tumor types in veterinary and human oncologic patients.^{9–11} In humans with non-small cell lung cancer, SLN mapping has been well described with a variety of preoperative and intraoperative techniques utilized successfully.^{12–15} Pulmonary SLN mapping has been studied in healthy dogs as both a model for human translation and for further consideration in clinical dogs. Techniques such as preoperative CT-lymphography, intraoperative scintigraphy, and intraoperative visible dye, have been described for pulmonary tissue in dogs without tumors.^{7,16,17} However, to date, SLN mapping has not yet been reported in dogs with primary lung tumors.

The objective of this case report was to describe the use of SLN mapping in a dog with a primary pulmonary carcinoma that underwent lung lobectomy via a minimally invasive approach. As minimally invasive techniques for pulmonary tumor resection in dogs become more widespread, development of intraoperative SLN

mapping techniques that can be performed minimally invasively, as well as via open surgery, will be important, and the technique described in this report allows for utility in a variety of approaches.

2 | MATERIALS AND METHODS

A 9-year-old female spayed Labrador retriever was presented to the Cornell University Hospital for Animals emergency service for referral for a pulmonary mass. The owners had noted progressive coughing and exercise intolerance of several weeks' duration. Thoracic radiographs performed by the dog's primary veterinarian identified a solitary pulmonary mass, and at the time of the veterinary visit, hemoptysis was also observed. On presentation to the emergency service at Cornell University, the dog was hemodynamically stable with a nonproductive cough. Complete blood count and biochemistry panel revealed an inflammatory leukogram with 23.1×10^3 neutrophils/ μL (reference range [RR]: 2.7–9.4) and slight elevation of alkaline phosphatase (117 U/L; RR: 7–115). The dog underwent thoracic and abdominal contrast-enhanced helical CT (2.0 mm slices; 16-slice Aquilion LB, Canon Medical Systems USA Inc., Tustin, California) under sedation with dexmedetomidine 6 $\mu\text{g/kg}$ and methadone 0.2 mg/kg IV. This revealed a solitary, 6.5 cm maximal diameter pulmonary mass in the right caudal lobe (Figures 1 and 2). Mild lymphadenopathy of the sternal ($1.3 \times 0.6 \times 0.6$ and $1.4 \times 1.0 \times 0.7$ cm) and central tracheobronchial ($1.2 \times 1.1 \times 1.1$ cm) lymph nodes was noted; the right tracheobronchial ($0.8 \times 0.8 \times 0.7$ cm) and left tracheobronchial ($1.0 \times 0.9 \times 0.6$ cm) lymph nodes appeared within normal limits (Figure 3). No substantial abdominal disease was identified.

The dog subsequently underwent general anesthesia for video-assisted thoracoscopic surgery (VATS) with lung lobectomy and concurrent SLN mapping and extirpation. Prior to anesthesia, the dog was administered pantoprazole 1 mg/kg IV and maropitant 1 mg/kg IV. The dog was premedicated with dexmedetomidine 10 $\mu\text{g/kg}$ IV and fentanyl 5 $\mu\text{g/kg}$ IV. After preoxygenation, anesthetic induction was achieved with ketamine 3.3 mg/kg IV and propofol 2.6 mg/kg IV (titrated to effect). After placement of an appropriately sized endotracheal tube, anesthesia was maintained with isoflurane delivered in 100% oxygen, and the dog was maintained on IV constant rate infusions (CRIs) of ketamine, fentanyl, and propofol as well as IV

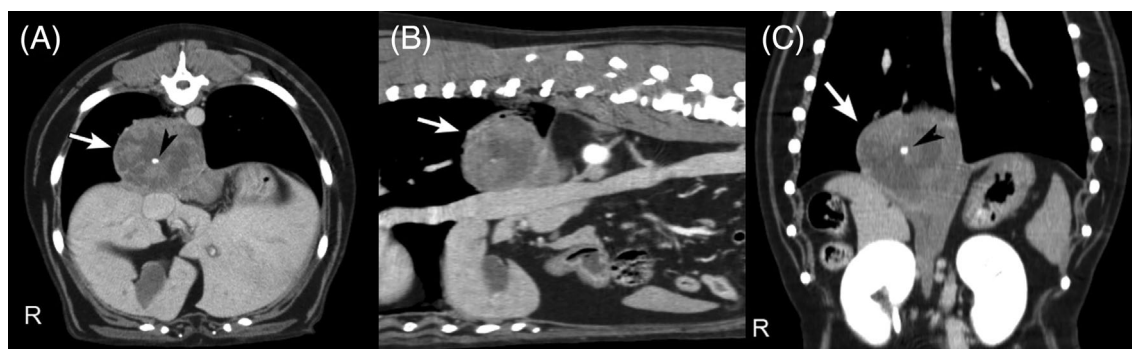


FIGURE 1 Post-contrast computed tomography images of the right caudal lung lobe mass (white arrow) displayed in a soft tissue window (WL 44/WW 372) as axial (A), sagittal (cranial-caudal orientation) (B), and dorsal (C) multiplanar reconstructions. The large lung mass is heterogeneously contrast enhancing with a small, central focus of mineral attenuation (black arrowhead).



FIGURE 2 A volume-rendered three-dimensional image of the right caudal lung lobe mass.

isotonic crystalloids (Plasmalyte). An epidural catheter was placed with aseptic technique and a total volume of 0.2 mL/kg (6.1 mL) of 0.125% ropivacaine, 5 µg/mL fentanyl, and saline was administered epidurally. Cefazolin 22 mg/kg IV was administered preoperatively and every 90 min perioperatively.

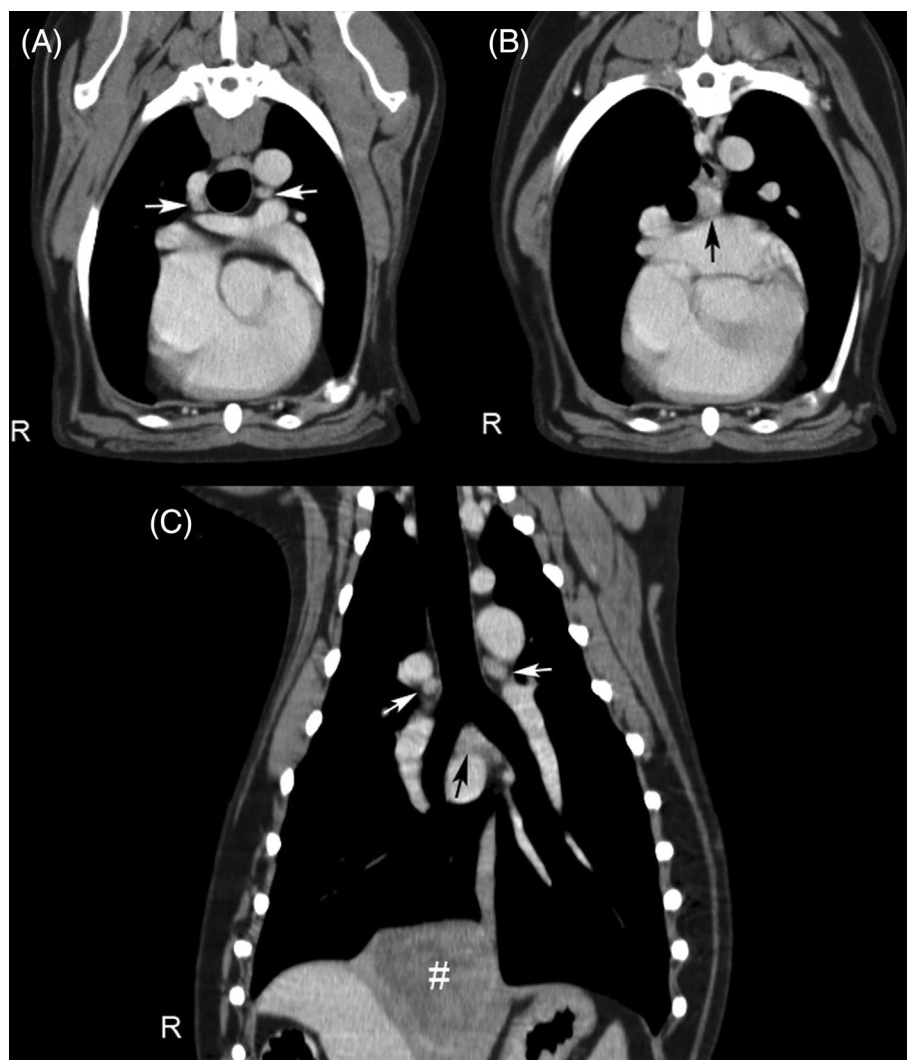
The dog was positioned in left lateral oblique recumbency, and the right thorax was clipped, prepared, and draped using a standard aseptic technique. A thoracoscopic approach was performed via three 5 mm cannulas placed in the right 4th intercostal space for the introduction of a 5 mm, 30°, near-infrared (NIR) capable telescope (IMAGE1 S Rubina, Karl Storz, Tuttlingen, Germany), blunt probe, and L-hook monopolar electro-surgical tip. One-lung ventilation was performed via bronchoscopic-guided placement of an endobronchial blocker within the right bronchus. The right caudal lung lobe mass was identified thoracoscopically, and the lung lobe was retracted ventrally with a blunt probe.

Monopolar electrosurgery was used to transect the pulmonary ligament of the right caudal lung lobe. Following mobilization of the lung lobe, 1 mL of 2.5 mg/mL indocyanine green (ICG) (HUB Pharmaceuticals, LLC, Scottsdale, Arizona) was administered percutaneously with a long 22 gauge needle into the right caudal lung lobe peritumoral tissues, aiming for injection into the grossly normal pulmonary tissue immediately adjacent to the gross mass, via a single injection under thoracoscopic guidance (Figure 4). Subsequently, an approximately 8 cm incision was made over the right 6th intercostal space. A wound retractor (Alexis O protector-retractor, Applied Medical, Rancho Santa Margarita, California) was inserted into the thorax through this incision. The right caudal lung lobe was mobilized but could not be exteriorized through the wound retractor prior to transection of the hilus due to the large size of the pulmonary mass. A vessel loop was passed around the right caudal lung lobe hilus to isolate and retract the hilus. The hilus was stapled and transected using two tan/vascular, 60 mm, Tri-Staple EndoGIA staplers (Covidien/Medtronic, Dublin, Ireland) in linear succession. Thereafter, the right caudal lung lobe and mass were exteriorized and removed from the chest through the wound retractor.

3 | RESULTS

Immediately following lung lobectomy, and approximately 10 min following peritumoral ICG injection, the 5 mm telescope was inserted through the wound retractor and used to image the right hemithorax. An overlay image of NIR on visible light was applied to assess for fluorescent lymphatics and lymph nodes secondary to peritumoral ICG injection. The right tracheobronchial lymph node, as well as a lymphatic tract entering the lymph node, appeared fluorescent (Figure 5). This lymph

FIGURE 3 Representative post-contrast computed tomography images of the right and left tracheobronchial lymph nodes (white arrows) and the central tracheobronchial lymph node (black arrow) displayed in a soft tissue window as (WL 44/WW 372) axial (A and B) and dorsal (C) multiplanar reconstructions. The right caudal pulmonary mass (#) is included on the dorsal reconstruction.



node was subsequently extirpated under direct visual and NIR guidance via blunt dissection and monopolar electrosurgery (Video S1). No sternal lymph nodes were identified via thoracoscopic-assisted exploration with visible light or NIR imaging, and no lymph nodes or abnormal structures were detected in the mediastinum or region of the central tracheobronchial lymph node via telescope guidance with visible light, palpation, or NIR fluorescence.

No hemorrhage was observed from the lung lobectomy or lymph node extirpation site, and a leak test with sterile saline and breath holds revealed no leakage of air. The remainder of the intrathoracic structures evaluated were unremarkable. The wound retractor and cannulas were removed. Prior to closure, the thorax was lavaged, and a sponge count was performed. A thoracostomy tube was placed under visual guidance and secured to the skin. Thoracic closure was routine. The thoracostomy tube was aspirated to negative pressure prior to moving from the operating room. The right caudal lung lobe

margin of excision was marked with ink, and all excised samples were submitted for histologic evaluation. Histopathology revealed a completely excised grade 1 pulmonary adenocarcinoma with pyogranulomatous bronchointerstitial pneumonia of the adjacent non-neoplastic right caudal lung lobe and moderate lymphofollicular hyperplasia with sinus neutrophilia and histiocytosis of the right tracheobronchial lymph node.

Postoperatively, the dog was treated and monitored according to standard of care in the intensive care unit. The dog received carprofen 2.2 mg/kg subcutaneously once, methadone 0.2 mg/kg IV every 6 hours, Plasmalyte 60 mL/kg per day IV, trazodone 5 mg/kg orally every 8 hours as needed, and gabapentin 10 mg/kg orally every 8 hours as needed. One day postoperatively, the dog was eupneic, hemodynamically stable, and apparently comfortable, and the thoracostomy tube was removed due to a low volume of pleural effusion and air evacuation. However, 2 days postoperatively, epistaxis and respiratory effort were observed. Thoracic radiographs revealed

acute, moderate to severe, predominantly cranioventral, pulmonary consolidation, prioritized as pneumonia with alternative differential diagnoses including pulmonary hemorrhage and noncardiogenic pulmonary edema. Complete blood count revealed a mild nonregenerative anemia (hematocrit 32%; RR: 41–58), neutrophilia ($36.0 \times 10^3/\mu\text{L}$) with left shift (3.9×10^3 bands), and thrombocytopenia ($110 \times 10^3/\mu\text{L}$; RR: 186–545). Biochemistry panel revealed hypernatremia (159 mEq/L; RR: 143–150), hypokalemia (3.2 mEq/L; RR: 4.1–5.4), hyperchloremia (125 mEq/L; RR: 106–114), hypocalcemia (8.3 mg/dL; RR: 9.4–11.1), hypoalbuminemia (2.3 g/dL;

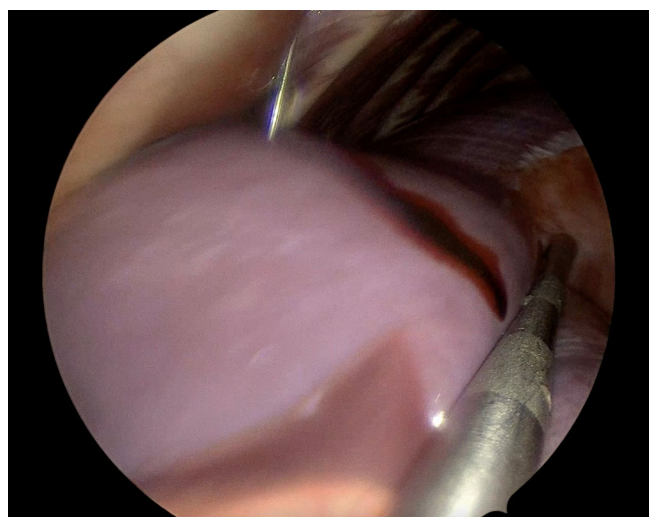


FIGURE 4 Percutaneous injection of 1 mL of 2.5 mg/mL indocyanine green (ICG) into peritumoral tissues of the right caudal lung lobe under thoracoscopic guidance using a long 22 gauge needle. A blunt probe stabilizes the right caudal lung lobe against the thoracic wall during injection. The lung lobe appears atelectatic due to one-lung ventilation, with a small amount of ICG visible extravasating from the needle puncture site.

RR: 3.2–4.1), hypoglycemia (67 mg/dL; RR: 68–104), elevated aspartate aminotransferase (AST) (340 U/L; RR: 18–56), and hyperbilirubinemia (0.6 mg/dL; RR: 0–0.2). Coagulation panel results were as follows: activated partial thromboplastin time (aPTT) 17.0 s (RR: 11.0–17.5), prothrombin time (PT) 13.0 s (RR: 11.0–15.5), fibrinogen 829 mg/dL (RR: 150–490), antithrombin 65% (RR: 65–145), and D-dimer 4,502 ng/mL (RR: 0–575). Thromboelastography results were as follows: tissue factor activated reaction time (TF-R) was 4.7 min (RR: 3–8), tissue factor activated maximum amplitude (TF-MA) was 64.6 mm (RR: 48–63), tissue factor activated alpha angle (TF-angle) was 67.7° (RR: 42–68), tissue factor activated kinetical time (TF-K) was 1.5 min (RR: 2–4), tissue factor activated lysis at 30 minutes (TF-LY30) was 0.3% (RR: 0–1), kinetical time (K) was 1.7 min (RR: 1.8–7.8), reaction time (R) was 5.8 min (RR: 5–14), tissue factor activated clot strength (TF-G) was 9.1 kdyne/s (RR: 4–8), tissue factor activated lysis at 60 minutes (TF-LY60) was 2.4% (RR: 0–6), maximum amplitude (MA) was 66.3 mm (RR: 47–60), alpha angle was 67.8° (RR: 25–65), lysis at 60 minutes (LY60) was 1.9% (RR: not established), lysis at 30 minutes (LY30) was 0.1% (RR: 0–1), and clot strength (G) was 9.8 kdyne/s (RR: 5–8). C-reactive protein was elevated (134.8 mg/L; RR: 0–12.0). The dog's clinical signs and anemia progressed, and the dog became oxygen dependent. Additional implemented treatments included aminocaproic acid 100 mg/kg IV every 6 hours, ampicillin/sulbactam 30 mg/kg IV every 8 hours, enrofloxacin 10 mg/kg IV every 24 hours, oxygen supplementation, and two packed red blood cell transfusions. The dog's cardiovascular status improved following the transfusions, and it maintained normal oxygenation on supplemental oxygen administration. However, the dog acutely collapsed and experienced

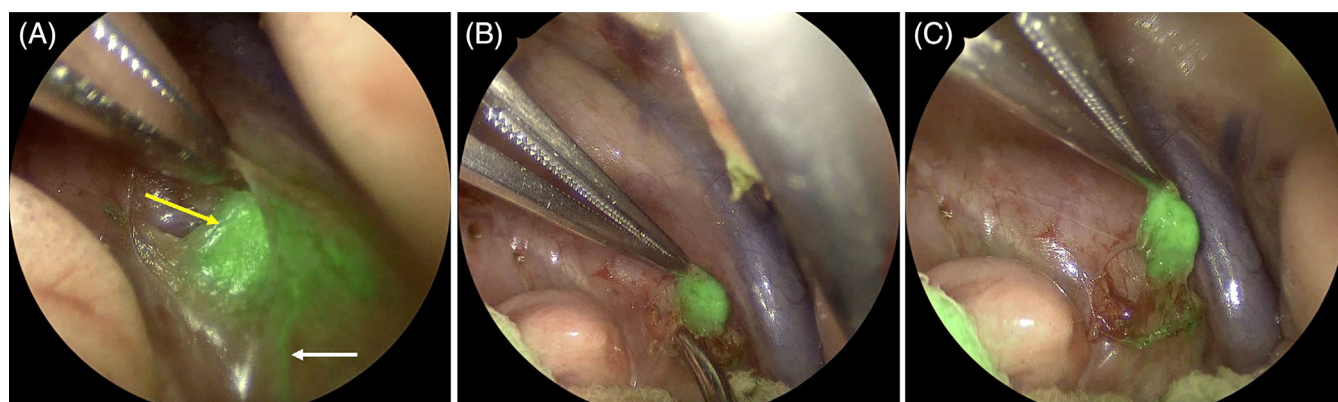


FIGURE 5 Images demonstrating fluorescence of the right tracheobronchial lymph node (yellow arrow in image A) as well as a lymphatic tract entering the lymph node (white arrow in image A). Images (B) and (C) demonstrate near-infrared image-guided dissection of the right tracheobronchial lymph node from its location deep to the mediastinal pleura.

cardiopulmonary arrest 3 days postoperatively; cardiopulmonary resuscitation was performed without return of spontaneous circulation. Necropsy was declined by the dog's owners.

4 | DISCUSSION

This manuscript represents the first case report documenting intraoperative SLN mapping for a primary pulmonary tumor in a dog. A VATS approach was utilized and deemed to be effective in allowing for mobilization of the pulmonary mass and lobe with thoracoscopic-guided ICG injection peritumorally. Between the ICG injection and the SLN mapping, a mini-thoracotomy approach was performed, and the lung lobe was excised. In humans with non-small cell lung cancer, SLN mapping is widely used with a variety of techniques for administration of ICG described, including peritumoral ICG injection followed by pulmonary mass excision and subsequent exploration and dissection of SLNs.¹⁵ Thus, a similar technique was utilized successfully in this dog. Other intraoperative ICG SLN mapping techniques that have been utilized in human non-small cell lung cancer include transbronchial injection, closure of injection sites, admixture with albumin, and dissection of the SLN prior to pulmonary tumor excision (but after allowing time for lymphatic flow to the SLN).^{12–14} However, the overall accuracy of SLN mapping appears to be excellent for a variety of techniques with ICG mapping for pulmonary tumors in people.¹² In this report on a dog with a pulmonary tumor, the technique described proved to be effective in identifying a SLN and guiding subsequent dissection without complications, although other techniques also warrant further evaluation. For cases in which a total thoracoscopic lung lobectomy is feasible, this technique may still be effective if ample time (approximately 5–10 min as described in people) is allowed for lymphatic flow from the lung lobe prior to lung lobectomy.

This report had several limitations. First, the technique described did not allow for simultaneous identification of all lymph nodes that could serve as sentinel for a pulmonary tumor, as only the right pleural space was entered and visualized. However, the only study to date using cross-sectional imaging with evaluation of all intrathoracic lymph nodes for SLN mapping of pulmonary tissue in healthy dogs identified the SLNs (tracheobronchial, pulmonary, and mediastinal lymph nodes) as either ipsilateral to the injected lung lobe or within the central tracheobronchial lymph node.¹⁷ Although the left-sided intrathoracic, mediastinal, and sternal lymph nodes could not be definitively identified with this right-sided VATS approach, the right tracheobronchial lymph node

exhibited strong fluorescence with afferent fluorescent lymphatic tracts. Also, no grossly enlarged lymph nodes or additional discernible fluorescent tracts or lymph nodes were observed in the region of the central tracheobronchial lymph node or in visible mediastinal or cranial intrathoracic structures, such that the right tracheobronchial lymph node was suspected to be sentinel for the pulmonary tumor in this dog. However, this limitation points to the need to further evaluate techniques, such as altered positioning and port placement, for SLN mapping in dogs with pulmonary tumors in order to enable simultaneous assessment of all intrathoracic lymph nodes that may serve as sentinel nodes for accurate staging. Another important limitation of this report includes the lack of long-term outcome data available due to the dog's systemic decline and arrest several days postoperatively. However, the nature of this dog's systemic compromise was not deemed to be associated with the SLN mapping procedure and no definitive surgical complications were identified, although necropsy was not performed.

In conclusion, the reported intraoperative SLN mapping technique utilizing ICG and NIR imaging was effective in identifying a SLN and allowing for safe, image-guided extirpation for subsequent histologic assessment and accurate staging in a dog with a primary pulmonary adenocarcinoma. The technique utilized via VATS in this study can be adapted for both open and total thoracoscopic procedures. Modifications of the SLN mapping technique to maximize efficacy and enable visualization of the entire potential lymphocentrum remain to be studied in dogs. Future large-scale, properly powered studies utilizing SLN mapping are needed to determine an accurate incidence of nodal metastasis in dogs with primary pulmonary neoplasia as well as the effect of extirpation of occult/early metastatic lymph nodes on oncologic outcomes and indications for adjuvant therapy in these dogs.

AUTHOR CONTRIBUTIONS

Griffin MA, DVM, DACVS (Small Animal): Contributed to case management and all data acquisition and analysis; contributed to composition and review of the manuscript for publication. Todd-Donato AB, DVM, DACVR-DI, DACVR-EDI: Contributed to imaging data acquisition and analysis; contributed to composition and review of the manuscript for publication. Peterson N, DVM, MBE, DACVECC: Contributed to case management and data acquisition and analysis; contributed to composition and review of the manuscript for publication. Buote NJ, DVM, DACVS (Small Animal): Contributed to case management and all data acquisition and analysis; contributed to composition and review of the manuscript for publication.

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CONFLICT OF INTEREST STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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