

Abdominal lymphatic drainage after thoracic duct ligation and cisterna chyli ablation in clinically normal cats

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OBJECTIVE

To characterize abdominal lymphatic drainage in cats after thoracic duct ligation (TDL) and cisterna chyli ablation (CCA).

ANIMALS

7 purpose-bred research cats.

PROCEDURES

Baseline CT lymphangiography was performed. A popliteal lymph node was injected with iohexol, and images were acquired at 5-minute intervals for 15 minutes. Cats underwent TDL and CCA; methylene blue was used to aid in identifying lymphatic vessels. The CT lymphangiography was repeated immediately after and 30 days after surgery. All cats were euthanized and necropsied.

RESULTS

Results of baseline CT lymphangiography were unremarkable for all 7 cats. Only 5 cats completed the study. Leakage of contrast medium at the level of the cisterna chyli was seen on CT lymphangiography images obtained from all cats immediately after surgery. Evaluation of 30-day postoperative CT lymphangiography images revealed small branches entering the caudal vena cava in 2 cats, leakage of contrast medium into the caudal vena cava with no visible branches in 1 cat, and no contrast medium in the caudal vena cava in 2 cats. Contrast medium did not flow beyond the level of the cisterna chyli in any cat. Gross examination during necropsy revealed that all cats had small lymphatic vessels that appeared to connect to local vasculature identified in the region of the cisterna chyli.

CONCLUSIONS AND CLINICAL RELEVANCE

Abdominal lymphaticovenous anastomoses formed after TDL and CCA in cats. This would support use of these procedures for treatment of cats with idiopathic chylothorax, although additional studies with clinically affected cats are warranted. (*Am J Vet Res* 2019;80:885–890)

Chylous effusion within the thoracic cavity occurs secondarily in cats as the result of many causes, including trauma, neoplasia, cardiac disease, and lung lobe torsion.¹ Most often, a definitive cause is not identified, and chylothorax is deemed idiopathic. Medical management of chylothorax involving intermittent thoracentesis, use of low-fat diets, and administration of pharmaceuticals (eg, rutin) may be attempted but generally has been unsuccessful.²

Multiple surgical procedures have been evaluated in an attempt to provide successful resolution of chylothorax. The surgical techniques most frequently described for treatment of chylothorax in dogs² and cats^{2,3} are TDL, subtotal pericardiectomy, and pleural omentalization. The purpose of TDL is to redirect flow of chyle from the thorax and thoracic duct, which leads to the development of new extra-thoracic lymphaticovenous anastomoses.⁴ Anatomy of the thoracic duct of cats is widely variable, which

necessitates lymphangiography,^{5,6} intraoperative injection of methylene blue,⁷ preoperative administration of a high-fat meal, or a combination of these techniques to guide surgeons when performing TDL. Development of fibrosing pleuritis has been reported in cats secondary to chronic exposure to chyle.⁸ For this reason as well as to mitigate constrictive pericarditis as a cause of chylothorax, subtotal pericardiectomy is often combined with TDL.⁹ Omentalization of the thoracic cavity has been described as an adjunct to TDL and pericardiectomy in an attempt to drain chylous fluid from the thoracic cavity to the peritoneal cavity and aid in resolution of the condition. The rate for successful resolution of chylothorax in cats after surgery performed with variable combinations of the aforementioned techniques ranges from 20% to 75% in large-scale studies,^{10,11} compared with 60% to 100% in dogs.^{1,11,12}

Cisterna chyli ablation has been described in dogs¹ and cats.^{13,14} The cisterna chyli is a confluence of lumbar and intestinal lymphatic trunks that drain into the thoracic duct.⁶ Lymphatic hypertension has been proposed as a possible reason that TDL for treatment of

ABBREVIATIONS

CCA Cisterna chyli ablation

TDL Thoracic duct ligation

chylothorax may fail¹³; CCA may prevent lymphatic hypertension by allowing direct drainage of lymph into the abdominal cavity with subsequent formation of abdominal lymphaticovenous anastomoses. In a study¹³ of dogs, CCA disrupted chylous drainage to the thoracic duct, and there was formation of lymphaticovenous anastomoses within the abdomen. A comparison of treatments of clinically affected dogs found that CCA with TDL was superior to subtotal pericardiectomy with TDL for complete resolution of chylothorax.¹ Typically, the authors have performed combined TDL, CCA, and subtotal pericardiectomy in cats with idiopathic chylothorax, although the impact of CCA on lymphatic drainage in cats has not been determined.

Results of lymphangiography after TDL in cats have been described¹⁵; however, to the authors' knowledge, no studies have been conducted to evaluate results of lymphangiography after TDL combined with CCA. Given the poor prognosis for cats undergoing surgery for treatment of chylothorax, additional studies are warranted to characterize the impact of TDL with CCA on lymphatic drainage in this species so that recommendations can be made regarding the use of CCA in conjunction with TDL in cats.

The purpose of the study reported here was to evaluate by use of CT lymphangiography the development of abdominal lymphaticovenous anastomoses in cats after TDL and CCA. We hypothesized that TDL combined with CCA in cats would not result in the development of lymphaticovenous anastomoses within the abdominal cavity by 30 days after surgery.

Materials and Methods

Animals

Seven purpose-bred research cats were acquired for the study. Cats had negative results when tested for FeLV and feline infectious anemia. Cats were allowed a minimum of 7 days to acclimate to their surroundings before study activities began. A baseline physical examination, serum biochemical analysis, CBC, and urinalysis were performed on all cats. The study was approved by the University of Georgia Institutional Animal Care and Use Committee and complied with published guidelines for the care and use of laboratory animals.¹⁶

CT lymphangiography

Baseline CT lymphangiography of the thoracic duct of each cat was performed immediately before surgery; procedures were as described elsewhere.⁵ Cats were premedicated with buprenorphine (0.02 mg/kg, IM), midazolam (0.5 mg/kg, IM), and ketamine hydrochloride (5 mg/kg, IM). A catheter was placed in a cephalic vein, and anesthesia was induced with propofol (up to 4 mg/kg, IV, to effect). An endotracheal tube was inserted, and anesthesia was maintained with isoflurane in oxygen. Heart rate and blood pressure were monitored by means of Doppler ultrasonography.

Cats were positioned in ventral recumbency with the pelvic limbs extended caudally for CT lymphangiography. Precontrast CT images of the thorax and abdomen were obtained by use of a 64-slice helical scanner.^a All CT images were acquired in a caudal-to-cranial direction in a soft tissue algorithm at 120 kVp, 200 mA, and pitch of 0.8, with images reconstructed into 2-mm-thick slices. By use of ultrasonographic guidance, 1.5 mL of iohexol was injected into a popliteal lymph node with a 26-gauge needle at a rate of 1 mL/min. The left popliteal lymph node was injected first in all cats. The CT images were obtained immediately after injection and at 5-minute intervals until 15 minutes after injection. The CT images were transferred to a workstation and evaluated with post-processing viewing software^b by a board-certified veterinary radiologist (SAS). When the initial injection of the popliteal lymph node did not result in adequate visualization of lymphatic vessels cranial to the node, the contralateral popliteal lymph node was injected and CT imaging repeated. When injection of neither lymph node resulted in successful visualization, the cat was excluded from the study and allowed to recover from anesthesia. Cats for which baseline CT lymphangiography images were successfully obtained remained anesthetized and were immediately prepared for surgery.

Immediately after surgery was completed, CT lymphangiography was repeated as described, with injection of contrast medium into the contralateral popliteal lymph node from the one used for the baseline image acquisition. After CT images were obtained at 15 minutes, isoflurane administration was discontinued. The cats received pure oxygen for at least 10 minutes before they were extubated.

All cats underwent repeated CT lymphangiography at (mean \pm SD) 30 \pm 3 days after surgery. The same anesthetic and CT lymphangiography procedures were used.

Surgery

The left lateral aspect of the thorax and abdomen of the anesthetized cats were clipped; clipping extended across the midline. Cats then were moved into an adjacent operating suite; they were positioned in right lateral recumbency, and a small towel was placed under each cat to slightly elevate the vertebral column. The left lateral aspect of the thorax and abdomen were aseptically prepared, and standard 4-corner draping of each cat was performed.

All surgeries were performed by a single board-certified veterinary surgeon (JAG). Similar to a previously described approach,¹⁷ an incision was made in the left 12th intercostal space, and the diaphragm was incised parallel to the 13th rib. This permitted access to both the thoracic duct and cisterna chyli. Stay sutures were placed in the diaphragm to aid in manipulation. Methylene blue (1% solution) was diluted 1:20 in sterile saline (0.9% NaCl) solution, and approximately 1 mL of the diluted solution was injected

into an ileocolic lymph node to aid in identification of the cisterna chyli and thoracic duct. The thoracic duct and any collateral structures detected in the caudal portion of the thorax were double ligated with 4-0 polypropylene. After TDL was completed, sharp excision was used to ablate all visible cysternal membranes and lymphatic connections up to the level of the diaphragm.⁴

An 8F red rubber catheter was placed in the thoracic cavity to allow for evacuation of air after closure of the diaphragm. A local intercostal block (0.5% bupivacaine; 1 mg/kg divided evenly between the 12th and 13th ribs) was administered, and the diaphragm then was closed by use of 2-0 polydioxanone in a continuous pattern with intermittent circumcostal passes. The thoracic cavity was closed by use of 3-0 polydioxanone with simple continuous circumcostal sutures. Musculature was closed by use of 3-0 polydioxanone in a simple continuous pattern, and subcutaneous tissues were closed by use of 4-0 polydioxanone in a simple continuous pattern. The thoracostomy tube was aspirated until negative pressure was obtained; it then was removed from the chest cavity. The skin was closed by use of 3-0 nylon with interrupted cruciate sutures.

Postoperative management

A fentanyl patch (which released a dose of 3 to 5 µg/kg/h) was placed on the lateral body wall of each cat at the end of surgery; the patch was allowed to remain in place for 5 days. Buprenorphine (0.02 mg/kg, IM or topical application to the buccal mucosa) was administered every 8 hours until signs of pain were adequately managed by use of the fentanyl patch alone. Cats were assigned a pain score by use of a previously described¹⁸ scoring system every 8 hours for the first 24 hours after surgery, then every 12 hours for 5 days after surgery. Cats with a total pain score > 8 or a pain score > 3 for any category were provided supplemental analgesia (buprenorphine; 0.02 mg/kg, IM or topical application to the buccal mucosa).

Cats were evaluated every 1 to 2 hours for at least 12 hours after surgery, every 8 hours on day 1 after surgery, and at least every 12 hours thereafter. When an increase in the respiratory rate paired with a decrease in lung sounds was detected in any cat, thoracocentesis was performed. Food and water were offered beginning 12 hours after surgery. Appetite, urination and defecation habits, and general demeanor were assessed every 12 hours for the first 5 days after surgery.

Necropsy

After the CT images were obtained on day 30 after surgery, each cat (while still anesthetized) was euthanized by injection of pentobarbital (1 mL/kg, IV). Necropsy of each cat was performed by the authors. After gross examination of the abdomen was completed, approximately 1 mL of methylene blue (1% solution) diluted 1:20 in sterile saline solution

was injected into an ileocolic lymph node to aid in identification of the region of the cisterna chyli before ablation, newly formed lymphatic vessels, and the thoracic duct.

Data analysis

A Kolmogorov-Smirnov normality test was performed on the variables age and body weight. Mean ± SD or median and range were reported, as appropriate.

Results

A total of 7 cats were initially enrolled in the study; 2 were excluded because evaluation of CT lymphangiography images did not reveal contrast medium in the thoracic duct 15 minutes after injection. For those 2 cats, injection of both popliteal lymph nodes did not result in successful visualization of the thoracic duct.

Five cats completed the study. All 5 were castrated males (mean ± SD age, 19 ± 5 months; mean body weight, 5.6 ± 0.6 kg). Results of the baseline physical examination, biochemical analysis, CBC, and urinalysis were unremarkable for all 5 cats.

Baseline CT lymphangiography was performed for all 5 cats. In 2 cats, injection of the left popliteal lymph node failed to yield contrast medium outside the node, but injection of the right popliteal lymph node successfully resulted in spread of the contrast medium. Contrast medium filled the cisterna chyli and thoracic duct in all 5 cats. Contrast medium reached the thoracic duct immediately after injection in 2 cats, within 5 minutes after injection in 2 cats, and within 15 minutes after injection in 1 cat. In 2 cats, the thoracic duct divided into 2 branches. In one of these cats, the branches started at the level of T7 and coursed cranially on both sides of the aorta and trachea, ending at the level of the first ribs; in the other cat, the branches were at the level of T10 to T11 and T7 to T9 (**Figure 1**). One cat had large (11 to 13 mm) inguinal lymph nodes.¹⁹ No other abnormalities were identified during evaluation of the baseline CT lymphangiography images.

Surgery was completed without complications in all 5 cats, and CT lymphangiography was performed immediately after surgery in all 5 cats. In 1 cat, injection of the right popliteal lymph node failed to yield contrast medium outside the node, but injection of the left popliteal lymph node resulted in successful spread of the contrast medium. In all 5 cats, contrast medium was evident at the level of the cisterna chyli, where it then leaked into the retroperitoneal space; this occurred immediately after injection in 3 cats and within 5 minutes after injection in 2 cats (**Figure 2**). No contrast medium was detected in the thoracic duct of any cat.

All cats recovered from surgery without complications. None of the cats required supplemental analgesia to the fentanyl patch after the first 24 hours. No complications were noted in any cat during the 30 days after surgery.

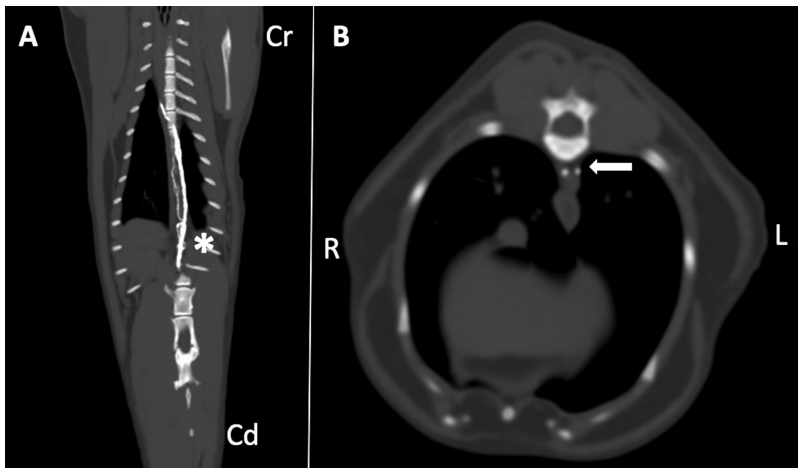


Figure 1—Maximum-intensity CT images (bone window) in the dorsal (A) and transverse (level of T10; B) planes of a cat after injection of contrast medium into a popliteal lymph node immediately before TDL and CCA. In panel A, the caudal extent of thoracic duct branching is indicated (asterisk). In panel B, 2 thoracic duct branches are evident dorsal to the aorta (arrow). Cd = Caudal. Cr = Cranial. L = Left. R = Right.

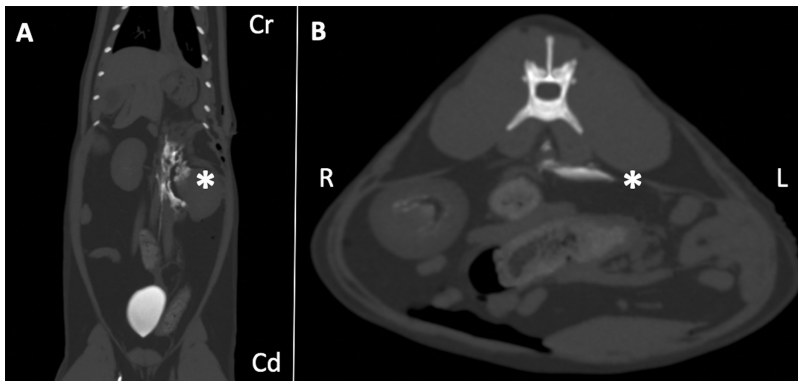


Figure 2—Maximum-intensity CT images (bone window) in the dorsal (A) and transverse (level of the right kidney; B) planes of a cat immediately after TDL and CCA. In both panels, notice leakage of contrast medium from the cisterna chyli (asterisk). See Figure 1 for remainder of key.

All cats were anesthetized approximately 30 days after surgery for CT lymphangiography. In 3 cats, the first popliteal lymph node (the left popliteal lymph node in 2 cats and the right popliteal lymph node in 1 cat) injection failed to yield contrast medium outside the node, but injection of the contralateral popliteal lymph node yielded successful spread of the contrast medium. Two of these 3 cats did not have spread of contrast medium from the same node for the first set of images obtained with CT lymphangiography. In the other cat, no problems were encountered with spread of contrast medium after injection of either popliteal lymph node for the first set of images obtained with CT lymphangiography. In the other 2 cats, the right popliteal lymph was injected, which resulted in successful spread of contrast medium.

Flow of contrast medium ended at the level of the cisterna chyli in all cats; no contrast medium entered the thoracic duct in any cat. In 1 cat, several small branches arose at the level of the right kidney,

and contrast medium entered the caudal vena cava immediately after injection (**Figure 3**). In 1 cat, multiple tubular soft tissue opacities were detected in the region of the cisterna chyli and caudal vena cava; however, contrast medium did not flow beyond this region by 15 minutes after injection. In 1 cat, contrast medium entered the caudal vena cava at the level of the cisterna chyli immediately after injection; however, no branches were identified. In the remaining 2 cats, contrast medium reached the level of the cisterna chyli immediately after injection, but no branches or entrance of contrast medium into the caudal vena cava was detected.

Necropsy revealed that all cats had omental adhesions over the site of the cisterna chyli. Sutures at the site of TDL were intact in all cats. Small lymphatic vessels were grossly visible in the region of the cisterna chyli and extended to the caudal vena cava in all cats; however, a defined cisterna chyli was not observed. Injection of methylene blue into an ileocolic lymph node did not result in any of the dye cranial to the site of the cisterna chyli in any cat. The caudal vena cava and vessels over the exposed kidney subjectively became bluer after dye injection.

Discussion

All cats had grossly visible formation of new lymphatic vessels in the region of the cisterna chyli 30 days after TDL and CCA. In 2 cats, definitive lymphaticovenous anastomoses were not identified during evaluation of CT lymphangiography images obtained 30 days after surgery; however, neither contrast medium (CT lymphangiography) nor methylene blue (gross examination during necropsy) was identified in the thoracic duct after injection of the lymphatic system. Formation of lymphaticovenous anastomoses within the abdominal cavity should prevent backflow to the site of TDL, which would further encourage redirection of lymph away from the thoracic cavity. These results suggested failure to form new lymphaticovenous anastomoses may not be a reason that the clinically reported prognosis in cats is worse than in dogs after TDL and CCA. Additional studies with clinically affected cats are needed to determine the success of these procedures, especially for cats for which surgical management does not resolve the condition.

Despite the fact that lymphaticovenous anastomoses were definitively identified within the abdomen of each cat during gross macroscopic exami-

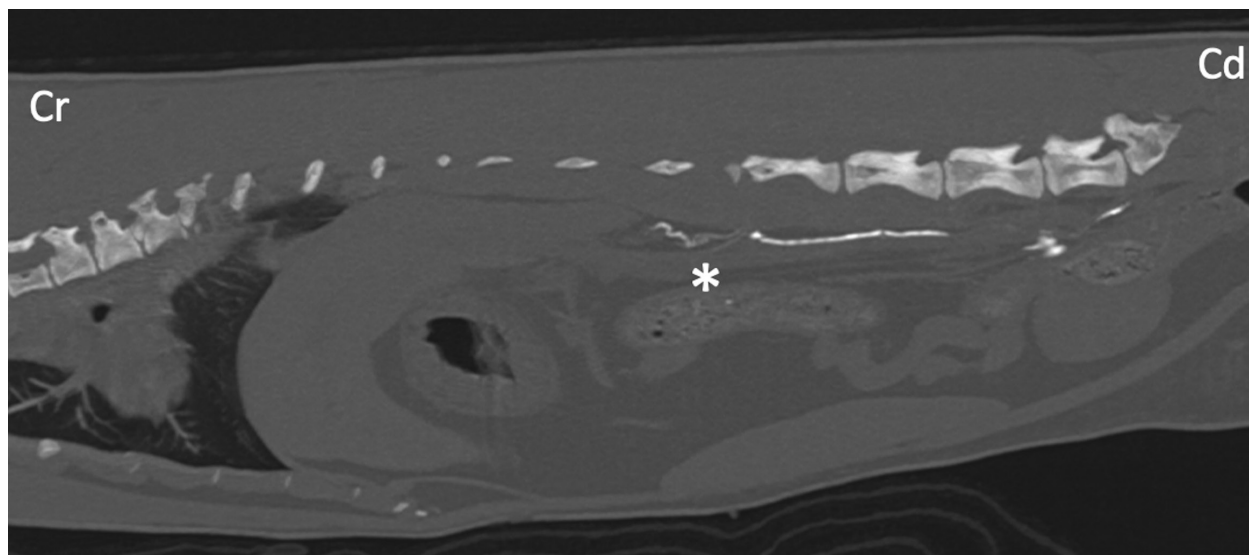


Figure 3—Maximum-intensity CT image in the sagittal plane of a cat 30 days after TDL and CCA. Slice thickness was 2 mm. Newly formed lymphatic branches are evident from the level of the right kidney to the caudal vena cava. Entry point of the new lymphatic branches into the caudal vena cava is indicated (asterisk). See Figure 1 for remainder of key.

nation, new lymphatic branches were identified in only 3 of 5 cats by use of CT lymphangiography. In several cats, injection of both popliteal lymph nodes resulted in successful flow of contrast medium into the abdominal cavity from only one of the lymph nodes. The new lymphatic branches were extremely small, compared with the size of the caudal vena cava. Given that small-diameter tubes have higher resistance than large-diameter tubes, these branches may have required more volume or greater pressure to adequately fill the new lymphatic branches, which possibly could explain the lack of our ability to identify these branches on the CT images of 2 cats. More volume or greater pressure potentially could be achieved by dilution of contrast medium with saline solution to obtain a larger volume.

Although the popliteal lymph node may drain to the abdomen in most cats, it is possible other lymph nodes (eg, sacral lymph nodes) may need to be targeted in some cats. Differences in anatomy may also explain the reason that some cats had unsuccessful results for CT lymphangiography after injection of one popliteal lymph node but successful results for CT lymphangiography after injection of the contralateral popliteal lymph node. However, the lymph node that yielded successful spread of contrast medium was not always consistent between the immediate postoperative CT lymphangiography images and those obtained 30 days after surgery. Thus, other mechanisms should be investigated to determine the best method for performing CT lymphangiography to enable evaluation of the thoracic duct anatomy in cats.

The reason for unpredictable resolution of idiopathic chylothorax of cats after surgical treatment remains unclear. An unidentified cause that leads to obstruction of outflow through the thoracic duct or leakage from the thoracic duct cranial to the site of

TDL is possible. Given the high sensitivity of cats to chyle within the thorax, it is also possible that chronic inflammation and fibrosing pleuritis play a role, even in affected cats that apparently are treated early in the disease process.⁸ Differences in abdominal fibrinolysis between dogs and cats may lead to differences in lymphaticovenous anastomosis formation in clinical cases. Additional studies to critically evaluate use of CT lymphangiography and management of cats with idiopathic chylothorax are warranted.

The study reported here had several limitations. The most notable were the small sample size and the use of clinically normal cats. Although new lymphaticovenous anastomoses formed in this group of healthy cats, it is possible the response in cats with naturally occurring idiopathic chylothorax may not be as robust. Use of the popliteal lymph node for injection and subsequent CT lymphangiography in the present study was associated with variable success, with results of the final CT lymphangiography matching necropsy findings in only 3 of 5 cats. Necropsy results were subjective and not performed by a board-certified veterinary pathologist. Additional studies that include a larger number of clinically affected cats with postoperative CT lymphangiography images should be conducted to confirm the findings reported here, especially given the variability detected for these 5 cats.

In the present study, all 5 healthy cats formed new abdominal lymphaticovenous anastomoses by 30 days after TDL and CCA. This suggested that these are viable surgical techniques for patients with chylothorax. Formation of new lymphaticovenous anastomoses in the abdominal cavity after CCA may be beneficial to cats with idiopathic chylothorax because they could direct fluid away from the thoracic cavity and prevent thoracic duct hypertension after TDL. Injection of a popliteal lymph node did not yield

successful spread of contrast medium in all cats. Additional studies to evaluate alternative CT lymphangiography techniques for affected cats with chylothorax are warranted.

Acknowledgments

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Footnotes

- a. Somatom Sensation, Siemens, Erlangen, Germany.
- b. Osirix, version 5.7, Pixmeo, Geneva, Switzerland.

References

1. McAnulty JF. Prospective comparison of cisterna chyli ablation to pericardectomy for treatment of spontaneously occurring idiopathic chylothorax in the dog. *Vet Surg* 2011;40:926-934.
2. Birchard SJ, McLoughlin MA, Smeak DD. Chylothorax in the dog and cat: a review. *Lymphology* 1995;28:64-72.
3. Fossum TW. Chylothorax in cats: is there a role for surgery? *J Feline Med Surg* 2001;3:73-79.
4. Hayashi K, Sicard G, Gellasch K, et al. Cisterna chyli ablation with thoracic duct ligation for chylothorax: results in eight dogs. *Vet Surg* 2005;34:519-523.
5. Lee N, Won S, Choi M, et al. CT thoracic duct lymphography in cats by popliteal lymph node iohexol injection. *Vet Radiol Ultrasound* 2012;53:174-180.
6. Kim M, Lee H, Lee N, et al. Ultrasound-guided mesenteric lymph node iohexol injection for thoracic duct computed tomographic lymphography in cats. *Vet Radiol Ultrasound* 2011;52:302-305.
7. Martin RA, Barber DL, Richards DLS, et al. A technique for direct lymphangiography of the thoracic duct system in the cat. *Vet Radiol Ultrasound* 1988;29:116-121.
8. Fossum TW, Evering WN, Miller MW, et al. Severe bilateral fibrosing pleuritis associated with chronic chylothorax in five cats and two dogs. *J Am Vet Med Assoc* 1992;201:317-324.
9. Fossum TW, Mertens MM, Miller MW, et al. Thoracic duct ligation and pericardectomy for treatment of idiopathic chylothorax. *J Vet Intern Med* 2004;18:307-310.
10. Fossum TW, Forrester SD, Swenson CL, et al. Chylothorax in cats: 37 cases (1969-1989). *J Am Vet Med Assoc* 1991;198:672-678.
11. Bussadori R, Provera A, Martano M, et al. Pleural omentalisiation with en bloc ligation of the thoracic duct and pericardectomy for idiopathic chylothorax in nine dogs and four cats. *Vet J* 2011;188:234-236.
12. Stewart K, Padgett S. Chylothorax treated via thoracic duct ligation and omentalization. *J Am Anim Hosp Assoc* 2010;46:312-317.
13. Sicard GK, Waller KR, McAnulty JF. The effect of cisterna chyli ablation combined with thoracic duct ligation on abdominal lymphatic drainage. *Vet Surg* 2005;34:64-70.
14. Thiel C, Held S, Kramer M. Cisterna chyli ablation in three cats with idiopathic chylothorax. *Tierarztl Prax Ausg K Kleintiere Heimtiere* 2013;41:221-228.
15. Kerpsack SJ, McLoughlin MA, Birchard SJ, et al. Evaluation of mesenteric lymphangiography and thoracic duct ligation in cats with chylothorax: 19 cases (1987-1992). *J Am Vet Med Assoc* 1994;205:711-715.
16. Institute for Laboratory Animal Research, National Research Council. *Guide for the care and use of laboratory animals*. Washington, DC: The National Academies Press, 2011.
17. Andrade N, Rivas LR, Milovancev M, et al. Intercostal approach for right adrenalectomy in dogs. *Vet Surg* 2014;43:99-104.
18. Schmiedt CW, Mercurio A, Vandenplas M, et al. Effects of renal autograft ischemic storage and reperfusion on intraoperative hemodynamic patterns and plasma renin concentrations in clinically normal cats undergoing renal autotransplantation and contralateral nephrectomy. *Am J Vet Res* 2010;71:1220-1227.
19. Perlini M, Bugbee A, Secrest S. Computed tomographic appearance of abdominal lymph nodes in healthy cats. *J Vet Intern Med* 2018;32:1070-1076.