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Perirectal injection of imaging materials for computed tomographic lymphography and near infrared fluorescent thoracoscopy in cats

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Abstract: For the treatment of chylothorax, the most common procedure is a thoracic duct ligation; however, recurrence of the issue is often common, generally due to the incomplete ligation of all thoracic duct tributaries. Therefore, accurate localisation of the thoracic duct tributaries is required to determine the ligation sites in each patient. The concept of the perirectal injection of the imaging materials, which provides a simple and minimally invasive approach, is investigated for computed tomographic (CT) lymphography and near infrared fluorescent thoracoscopy in cats. Three clinically healthy cats were used for the CT lymphography, and two clinically healthy cats were used for the near infrared fluorescent thoracoscopy. Iodine contrast agent for the CT or indocyanine green for the thoracoscopy was injected subcutaneously into the peri-anal tissue. The injection site was massaged for 5 min post-injection. However, in the indirect injection of the imaging materials, in three iohexol-administered cats, the abdominal lymphatics, cisterna chyle, and thoracic duct could be depicted by the CT lymphography; and in both indocyanine green-administered cats, the thoracic duct was depicted running alongside the aorta by the near infrared fluorescent thoracoscopy. The ideal imaging procedure for the thoracic duct ligation involves the pre-operative CT lymphography of the entire pathway of the lymphatic vessels in advance, followed by the direct visualisation using a thoracoscopy. A combined CT lymphography and thoracoscopy could be a reliable method for successful surgeries. Crucially, the subcutaneous peri-anal injection of a contrast/dye provides a simple and minimally invasive method for the pre-operative and intra-operative depiction of the lymphatic pathways.

Keywords: computed tomography; contrast agent; indocyanine green; iohexol; iopamidol

Chylothorax is a condition caused by chyle leaking into and accumulating in the pleural space. Symptoms of chylothorax include respiratory impairment, weight loss, and exercise intolerance, however these symptoms are not specific for chylothorax (Fossum and Mertens 2004). A definitive diagnosis can be provided through the confirmation of small lymphocytes from a fine needle aspiration and/or an increased triglyceride concentration in the pleural effusion. Several medical treatments are currently suggested for chylothorax; however, surgical management is generally recommended because medical treatments are often unable to completely resolve chylothorax. Surgical treatment for chylothorax includes thoracic duct ligation (Birchard et al. 1998), pleuroperitoneal shunting (Smeak et al. 2001), thoracic omentalisation (LaFond et al. 2002), cisterna chyli ablation (Hayashi et al. 2005), and pericardiectomy (Fossum and Mertens 2004). Thoracic duct ligation

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is the most commonly performed surgical procedure for the chylothorax treatment. However, even in cases in which the thoracic duct is ligated, a recurrence often occurs and is thought to be caused by a failure to completely occlude all the tributaries of the thoracic duct divisions (Bilbrey and Birchard 1994). Kagan and Breznock (1979) previously reported that seven patterns of thoracic duct tributaries exist in dogs; but those of cats were unknown. The accurate identification of the lymphatic branches minimises the risk of the ducts being missed during surgery. Therefore, to achieve successful thoracic duct ligation, the precise assessment of the thoracic duct tributaries is required to determine the optimal ligation site for the surgical planning in each individual chylothorax case.

Previous studies for lymphographic images by computed tomography (CT) were performed by the direct injection of contrast agents into the mesenteric lymph nodes via laparotomy in dogs (Johnson et al. 2009) or in cats (Kim et al. 2011). As with other minimally invasive procedures, the thoracoscopic approach provides another option. To identify the lymphatics, they are stained using dyes, which are injected into the mesenteric lymph nodes via a laparotomy. However, a laparotomy for the injection of mesenteric lymph nodes is an invasive procedure. In conventional lymphography of humans, dyes can be directly injected into the lymph canal of the dorsum of the foot (Kinmonth et al. 1955); however, this procedure is difficult in dogs/cats due to the small size of the lymph canals. An alternative method, is the direct injection of a contrast medium into the popliteal lymph nodes in dogs, but popliteal lymph nodes are often small with reproducible results difficult to obtain (Steffey and Mayhew 2018). This is likely the same situation for cats when attempting to obtain an effective contrast volume. To address these limitations, in this paper, the concept of the perirectal injection of materials, which is easy to approach and less invasive, is investigated for the CT and thoracoscopy in cats.

MATERIAL AND METHODS

Animals. Three clinically healthy female cats (age: 6–7 years, BW: 3.4–3.9 kg) were used for CT lymphography, and two clinically healthy female cats (age: 3 years, BW: 3.5–3.8 kg) were used for a near infrared fluorescent thoracoscopy. The experimental protocol for this study was approved by the Office of Laboratory Animal Welfare of Azabu University (No. 120528-3).

CT Lymphography. The animals were fasted for six hours prior to the start of the experiment. Anaesthesia was induced using butorphanol (Vetorphale®, Meiji Seika Pharma, Tokyo, Japan; 0.2 mg/kg, *i.v.*) and propofol (Rapinovet®, Schering-Plough, Tokyo, Japan; 6 mg/kg, *i.v.*), and maintained using 2% isoflurane inhalation (Forane®, Abbot, Tokyo, Japan). Iohexol (Omnipaque®, Daiichi-Sankyo, Tokyo, Japan; 300 mg I/ml) or iopamidol (Oypalomin®, Fuji Pharma, Toyama, Japan; 300 mg I/ml) warmed to 37 °C, was injected subcutaneously into peri-anal tissue by a 23-gauge needle. The injection doses were 1.2 or 1.8 ml/kg, with both dosage regimens used in each of the three cats in separate experiments separated by a minimum of one week. The total volume of the contrast media was administered slowly for 30 sec in duration, and the injection site was massaged for 5 min post-injection. The CT images were obtained in a supine position from the inlet of the thorax to the anus using a CT scanner (Asteion, Toshiba, Tokyo, Japan), with a voltage of 120 kV, amperage of 60 mA and slice thickness of 2.0 mm, at 5, 15, and 25 min post-injection. The field of view was adjusted for the size of the cats. The DICOM data were transferred to a viewer and three-dimensional images were generated using an image processing workstation (Virtual Place, AZE, Tokyo). The order of contrast administration was randomised, and the interval between the experiments was over one week. The contrast enhancement of the lymphatics for the CT was subjectively evaluated by discussion of the four investigators (K. K., M. O., N. I., M. A.), and scored three criteria such as 'Visualised all pathways from the administration site to the thoracic duct', 'Visualised most of the pathways from the administration site to the thoracic duct' and 'Not visualised'.

Near Infrared Fluorescent Thoracoscopy. A video endoscopic system (Karl Storz Endoskope Japan, Tokyo, Japan) was used for the near infrared fluorescent thoracoscopy. The fluorescent of this scope emits an excitation light wavelength of 805 nm, and receives a wavelength of 835 nm by the near infrared light. Indocyanine green (ICG, Dianogreen®, Daiichi-Sankyo, Tokyo, Japan; 2.5 mg/ml), a deep green colour, was used to identify the lymphatics.

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ICG is a safe and effective dye for operative imaging of the lymphatic system as it binds to plasma lipoproteins and is taken up by the lymphatic lumen (Steffey and Mayhew 2018). ICG is commonly used for hepatic function tests and in fundoscopic examinations, both of which have an extensive history in human medicine. The left side of the thorax was sterilised as the surgical field and a thoracic scope was placed in the 8–9 intercostal space under anaesthesia, using the same anaesthetic protocol described for the CT lymphography procedures. The ICG, warmed to 37 °C, was injected subcutaneously into the peri-anal tissue. The injection site was massaged for 5 min, and the thoracic cavity was observed continuously for 15 min post-injection. Prior to recovering from the anaesthesia, the cats were administered meloxicam (Metacam®, Boehringer Ingelheim, Tokyo, Japan; 0.1 mg/kg, *s.c.*) as a non-steroidal anti-inflammatory drug for a postoperative analgesia. Following the recovery from the anaesthesia, the cats were administered amoxicillin (Amoxiclear®, Kyoritsu Seiyaku, Tokyo, Japan; 20 mg/kg, *p.o.*) as an antibiotic, and robenacoxib (Onsir®, Elanco, Tokyo, Japan; 1.0 mg/kg, *p.o.*) as a non-steroidal anti-inflammatory drug for 1 week. Visualisation of the thoracic ducts for the thoracoscopy was subjectively evaluated by the discussion of two operators (K. K., E. K.) during the procedure, and decided if it was visible/invisible.

RESULTS

CT Lymphography

In all three cats administered with iohexol, the abdominal lymphatics, cisterna chyli, and thoracic duct could be depicted after five min; these contrast effects were still visible after 15 and 25 minutes. The subcutaneous peri-anal administered contrast agent was distributed cranially via the abdominal lymphatics beyond the oesophageal hiatus, bifurcated at the thoracic duct at the level of T13, continued cranially through the chest cavity along the dorsal side of the oesophagus, joined together again at the level of T7, and diverted ventrally at the mediastinum (Figure 1). Conversely, the contrast effects of iopamidol varied widely between the animals and were not reproducible (Table 1). In the 3-dimensional CT imaging with the iohexol, the whole pathway of the lymphatic was depicted (Figure 2). No adverse effects, including a skin inflammation or the ulceration at the administration site, were observed in any animal following either procedure.

Near Infrared Fluorescent Thoracoscopy

The subcutaneous perirectal injection of ICG in both cats resulted in the depiction of the thoracic

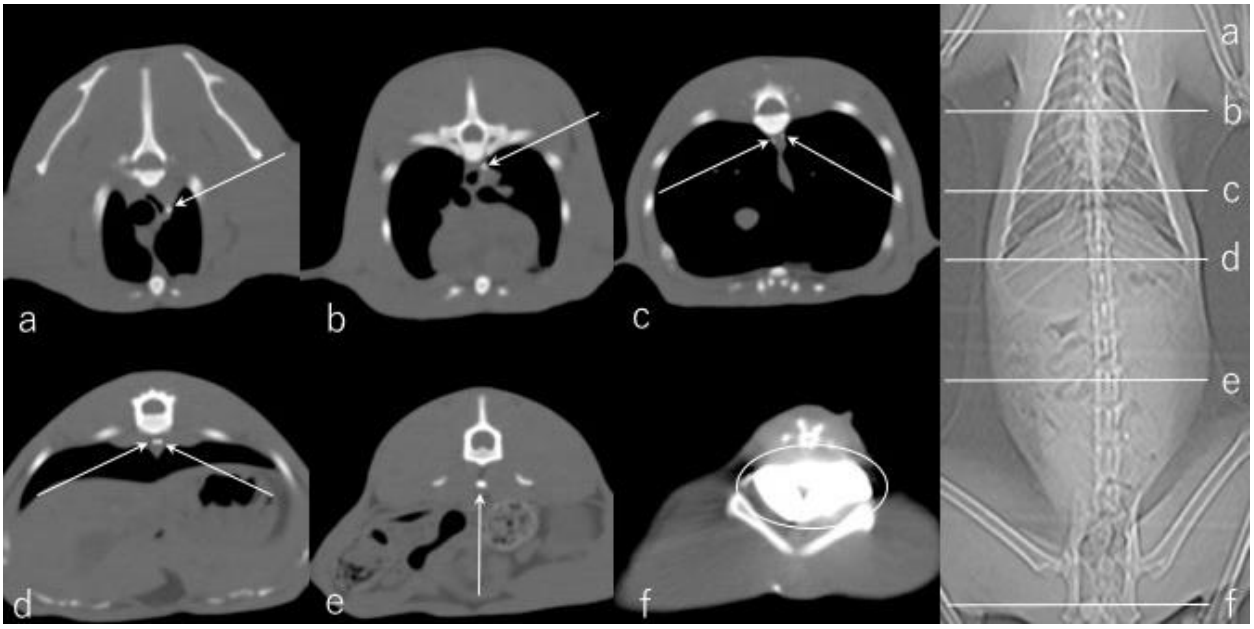

Figure 1. Transverse CT lymphographic images obtained five min after administration of iohexol (300 mg I/ml, 1.8 ml/kg). The subcutaneous injection site is shown with a circle in Figure 1f. The abdominal lymphatic vessels leading to the thoracic duct are depicted (arrows)

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duct running alongside the aorta through the near infrared fluorescence for the full 15-min observation period; conversely, the thoracic duct was difficult to visualise under the visible light (Figure 3). After the experiment, no cats experienced any adverse events, such as a skin inflammation or an ulcer, at the administration site. No adverse effects, such as an infection or dehiscence, were observed in either animal during the study.

Table 1. The comparison of the contrast effect on the lymph vessels between iohexol and iopamidol

Animal	Contrast agent	Dosage (ml/kg)	Minutes		
			5	15	25
A	lohexol	1.2	○	○	○
	lopamidol	1.2	×	×	△
B	lohexol	1.8	○	○	○
	lopamidol	1.8	×	△	×
C	lohexol	1.8	○	○	○
	lopamidol	1.8	△	○	×

○ = visualised all pathways from administration site to thoracic duct; △ = visualised most of all pathways from administration site to thoracic duct; × = not visualised

Figure 2. The three-dimensional CT lymphographic images obtained five min after administration of iohexol (300 mg I/ml, 1.8 ml/kg). The subcutaneous injection site and the abdominal lymphatic vessels leading to the thoracic duct are depicted

DISCUSSION

Our results demonstrated that the perirectal injection of a contrast agent/dye is useful for the visualisation of the lymphatics through both CT and thoracoscopy. Theoretically, materials with a molecular weight above 5000 are able to pass into the lymphatic lumen (Leak 1970), however, the molecular weights of iohexol, iopamidol, and ICG, the agents used in this study, are 821, 777, and 774, respectively. It is considered that the agents administered peri-rectally distribute around the rectal submucosa and infiltrate into the *follicu-lus lymphaticus*, then the materials might drain into the lymph lumen. Hayashi et al reported, in a study involving the submucosal injection in dogs, that the indirect injection of contrast agents could provide the sufficient contrast of lymph nodes (Hayashi et al. 2006), supporting our results. The dosages used in the present study are around 10 times greater for the iodinated contrast media and around 30 times greater for the ICG than in a previous study in dogs involving direct injection into the popliteal lymph node (Steffey and Mayhew 2018). Human case studies involving the indirect administration

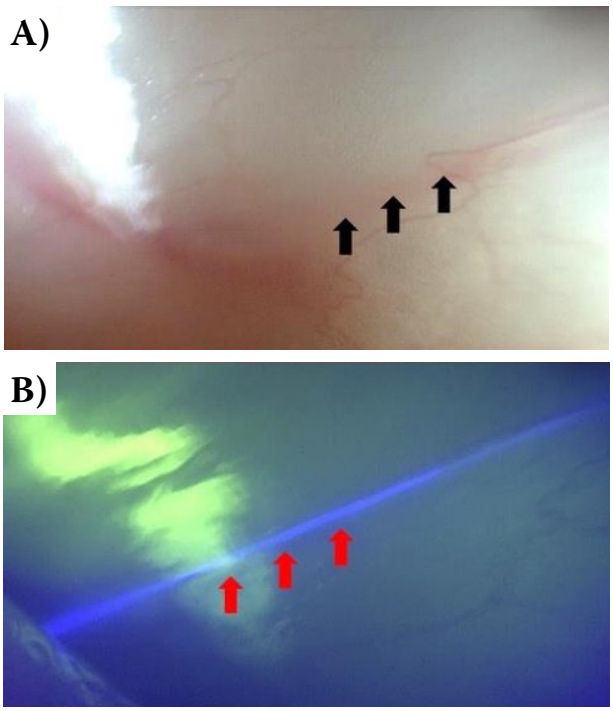

Figure 3. Visible light (**A**) and near infrared fluorescent thoracoscopic images (**B**) obtained 4 min after the subcutaneous injection of indocyanine green (1.8 ml/kg). The thoracic duct (arrows) is visible in the near infrared thoracoscopy image

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of contrast agents have also reported high dosages (Kamiya et al. 2009). Therefore, indirect injection can successfully provide the contrast for the lymphography due to the increased doses injected. Large doses of contrast agent cannot be administered in cats and dogs through direct injection into the popliteal lymph nodes due to their small size (Steffey and Mayhew 2018). Subcutaneous perianal tissue administration, as was employed in this study, can be performed more easily than a direct injection into the lymph node, with the loose tissue around the anus allowing for the administration of a higher dosage. The volumes administered through this method may be sufficient to reach the thoracic duct. The osmolarities of iohexol and iopamidol are 821 and 777 mOsm/kg H_2O, respectively. There is a concern over the subcutaneous injection of large volumes of high osmolarity iodine contrast agents. However, a high dosage of contrast agents was injected subcutaneously into the perianal tissue in this study, with no adverse effects, such as inflammation or ulceration, at the administration site.

The contrast effect of iohexol was superior to that of iopamidol in the CT lymphography. The contrast effect of iopamidol varied widely, meaning the results were not reproducible. Iohexol and iopamidol are both chemically similar water-soluble iodine contrast agents. The molecular weight of iohexol is slightly greater than that of iopamidol; however, neither is considered a large molecular weight agent, which is not considered to positively drain into the lymphatic lumen. The authors' speculation is that the differences observed in this study in the contrast effect between iohexol and iopamidol might be influenced by their differences in molecular weight.

It is known that the lymph fluid flows passively in the lymphatics, and that the indirect pressure from the muscular motion facilitates lymph flow. Massage at the injection site is, therefore, the key to the successful indirect lymphography (Ando et al. 2012; Favril et al. 2019). Passive motion was essential for the uptake into the lymphatic vessels and to promote the lymph flow.

A CT lymphography presents the advantage of depicting the entire lymphatic pathway prior to surgery. However, the contrast agent is clear and colourless, so it is impossible to observe it with the naked eye. Therefore, colourisation of the thoracic duct is essential during the thoracoscopy. In the present study, visible light was incapable of visualising the thoracic duct. Visible light penetrates only a few hundred microns into the tissues; however, the near infrared light of an 805 nm wavelength penetrates several millimetres into the tissues (Kitai et al. 2005; Ohnishi et al. 2005). The ICG could not be visualised with the visible light due to limited penetration depth through the peri-aortic fat. In addition, the near infrared fluorescent thoracoscopy presents the advantages of a real time observation and the magnification of the small structures. The identification of the thoracic duct enables the surgeons to ensure the complete ligation of the lymphatic branches, decreasing the risk of iatrogenic damage to the thoracic duct due to any misunderstanding (Steffey and Mayhew 2018). In the present study, the visibility of the thoracic duct during the thoracoscopy was maintained for 15 min, which is a sufficient period for the completion of the surgical procedure. Furthermore, the perirectal ICG injection is expected to be less invasive than the direct injection into the mesenteric lymph nodes via a laparotomy, potentially contributing to a reduced surgical time.

A thoracoscopy enables the direct observation of the thoracic duct, however, variability in the location of the thoracic duct between the animals means it may be difficult to locate it without the information obtained from the CT lymphography (Steffey and Mayhew 2018). Therefore, the ideal imaging method involves the prior observation of the full pathway of the lymphatic vessels via CT lymphography, followed by the direct observation using thoracoscopy. However, this study was limited to a trial in a small number of clinically healthy cats, therefore, further study is required to assess the application of such techniques in chylothorax cases. This study suggests that the combined CT lymphography and thoracoscopy could be a reliable tool for successful ligation. Furthermore, the subcutaneous, peri-anal injection of an iodine contrast/dye provides a simple and minimally invasive method for the pre-operative and intra-operative depiction of the lymphatic pathways.

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