Determination of Optimal Dosage and Delay Time for Computed Tomographic Lymphography after Percutaneous Injection of Iohexol into Popliteal Lymph Nodes in Dogs

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(Received 23 July 2008/Accepted 26 December 2008)

ABSTRACT. The purposes of this study were to determine the optimal dose and delay time for lymphography by injection of iohexol into popliteal lymph nodes and to assess images of computed tomography by the established protocol. Three different doses (30, 60 and 90 mgI/kg) of water-soluble iodinated contrast medium were injected into 15 popliteal lymph nodes of 10 adult beagles, and fluoroscopy was performed. Filling and duration of contrast media and the number of visible ducts from popliteal lymph nodes to the thoracic duct and its branches were recorded. CT lymphography was performed, and the number of visible thoracic ducts was compared with that found by radiographic lymphography. Radiographs obtained between 130 and 800 seconds after injection of contrast medium provided a detailed view of the thoracic duct. The dose of 60 mgI/kg was determined to enable quality diagnostic imaging without extranodal leakage in radiographic lymphography. There was no significant difference in the number of thoracic ducts between the two modalities at each anatomic location. However, CT lymphography provided images of the thoracic duct with better spatial resolution and without superimposition of surrounding tissue. The present study provides an adequate delay time and injection for identification of the canine thoracic duct, and therefore, this technique could be applied to diagnosis of disease associated with chest lymphatic drainage.

KEY WORDS: CT, dog, Percutaneous ultrasound guided popliteal lymphography, thoracic duct.

Chylothorax is a condition characterized by accumulation of a fluid containing chylomicrons in the pleural cavity. Lymphography is essential for evaluating the integrity of the thoracic duct and its branches, and several techniques, including pedal lymphography, and direct mesenteric lymphangiography, have been used to identify the causes of chylothorax in dogs. Direct lymphangiography may provide information about the causes of chylothorax [11]. In addition to diagnosis of chylothorax, failure to ligate all collateral branches of the caudal thoracic duct is the most common cause of operative failure. For this reason, lymphangiography just before and after ligation has been recommended to ensure complete ligation of the thoracic duct [6, 7].

Esterline et al. suggested that CT may be able to quantify the branches of the thoracic duct more accurately than standard radiographic lymphangiography; however, they injected the contrast media by laparotomy, and their method could not overcome the disadvantages of the long anaesthesia time and laparotomy [4].

Subsequently, a relatively non-invasive, simple and rapid lymphography with a high success rate was used in dogs with chylothorax. A study in 2006 demonstrated that percutaneous injection of contrast medium into popliteal lymph nodes can visualize the thoracic duct well [8]. However, further study was necessary in regard to the optimal dose of contrast media and delay time for radiographic and computed tomographic lymphography because the popliteal lymph node has a limited capacity for injection, and too large a dose of contrast media can result in long a injection time. There are also no reports of computed tomographic lymphography by injection of iohexol into popliteal lymph nodes.

The purposes of this study were to determine the optimal dose of iohexol and ascertain the delay time for radiographic delineation of the thoracic duct and its branches using popliteal lymph nodes and to compare images from computed tomography with radiographic images for identification of the thoracic duct.

MATERIALS AND METHODS

Animals: Lymphography was performed in one popliteal lymph node each for 15 adult Beagles. Five dogs were assigned to each of three groups, which were formed on the basis of the 30, 60 and 90 mgI/kg iohexol doses. After two weeks, 5 randomly selected dogs were used for computed tomographic lymphography by injection of iohexol into a popliteal lymph node. The Beagles were supplied from the animal facility of the Veterinary Medical Hospital at Chun-
gbuk National University and use of the animals in this study was approved by the office of Laboratory Animal Welfare. The body weights of the dogs ranged from 9 to 12 kg with a mean (± SD) of 10 (± 0.5) kg. The dogs were deemed healthy based on the absence of clinical signs, results of physical examination, serum biochemistry profile and complete blood cell count. Furthermore, fine needle aspiration of the popliteal lymph node was performed before lymphography to rule out the presence of lymphadenopathy.

Protocol for radiographic lymphography: Each dog was premedicated with atropine (Atropine®, Daewon Pharm., Republic of Korea). Anesthesia was induced with propofol (Anepol®, Hana Pharm., Republic of Korea) and maintained using isoflurane (Isoflurane®, Choongwae Pharma., Republic of Korea) after endotracheal intubation. During anesthesia, oxygen saturation, heart rate, end tidal CO2 and non-invasive blood pressure were monitored continuously. Following anesthesia, the dogs were placed into lateral recumbency on the fluoroscopy table. The popliteal lymph node image was obtained using a 7 MHz linear transducer (SonoAce 8800 MT, Medison, Republic of Korea). Under the guidance of ultrasonography, manual injection of three different doses (30, 60 and 90 mgI/kg) of water-soluble iodinated contrast medium (Omnipaque™ 300, Nycomed Inc., Princeton, NJ, U.S.A.) into 15 popliteal lymph nodes was performed using a 27 mm-gauge needle. Real-time observation of contrast medium flow was performed fluoroscopically, and still images of the thoracic duct and branches were taken and recorded in a computer. Filling time to the point at which the contrast medium began to opacify each lymph node, including the inguinal lymph node, external iliac lymph node, lumbar lymphatic duct, cisterna chyli and thoracic duct, and duration of opacification in each lymph node were recorded. The time at which the contrast media disappeared was defined as the time at which the lymphatic duct could no longer be differentiated from adjacent soft tissue structures.

After determination of the optimal dose of contrast medium through image quality evaluation, the computed tomographic scanning time for the thoracic duct was established based on its filling time.

Image evaluation of radiographic lymphography: Image quality evaluation for radiographic lymphography was carried out to determine the optimal dose of contrast media through blind testing at each dose, respectively, with the help of 3 veterinarians, who were not aware of the aim of this experiment. The parameters of still images were assessed on a computer monitor and included the overall quality of the depiction of the thoracic ducts. The images were assessed twice using a five point scale: a score of 1 meant unacceptable; a score of 2 meant suboptimal; a score of 3 meant adequate; a score of 4 meant good; and a score of 5 meant excellent diagnostic quality. Diagnostic quality was considered to be achieved when the score was 3 or higher.

Protocol for CT lymphography: Computed tomographic (CT IQ, Picker, Philips Medical Systems, Andover, MA, U.S.A.) scan of the entire thorax and the cranial abdomen, centered over the vertebra, was performed following injection of 60 mgI/kg of a contrast medium into the popliteal lymph node in five dogs under identical anesthetic procedures. After acquiring a scout series, a computed tomographic scan was performed from 118 seconds to 1022.8 seconds after injection of contrast media. The slice thickness of the images was 2 mm, with an interslice couch movement of 5 mm. Contrast medium was identified within the thoracic ducts and associated tributary lymphatic vessels, and the numbers of thoracic ducts confirmed was recorded on film.

Statistical Analysis: For selection of the optimal dose of contrast media, ANOVA was used to test for differences in the median scores of the doses. The mean image quality scores assigned to each dose were compared by variance analysis. If significant differences were found with this test, post hoc analysis was performed with a Tukey B test. Weighted kappa was used to measure the chance corrected interobserver and intrabserver reliability. While no absolute definitions were possible, we rated the strength of agreement with scores of 0.20 or less as ‘poor’, 0.21–0.40 as ‘fair’, 0.41–0.60 as ‘moderate’, 0.61–0.80 as ‘good’ and 0.81–1.00 as ‘very good’.

Comparison between numbers of thoracic ducts was performed for radiographic images and CT images using Wilcoxon rank sum analysis. Hypotheses were accepted if the P values were less than 0.05.

RESULTS

Radiographically, the popliteal lymph node, inguinal lymph node, external iliac lymph node, lumbar lymphatic vessels, cisterna chyli and thoracic ducts were delineated in all dogs after injection of the contrast medium. All radiographic images showed the thoracic duct and its branches cranial to the diaphragm, as well as anastomoses cranial to the heart.

Popliteal lymph nodes were found to be either round or oval in shape depending on the dog; however, it was difficult to identify an inguinal lymph node in all the dogs. The number of thoracic ducts identified on radiographs after lymphography varied from one to four depending on the individual. Lymphatic vessels were clearly opacified, and there were several lymphatic vessels connecting the lymph nodes (Fig. 1).

Lymph nodes and lymphatic vessels were identified in radiographic imaging at all doses of contrast medium. However, the higher dose of contrast medium resulted in a higher score (Table 3). However, leakage of the contrast medium around the popliteal lymph node at a dose of 90 mgI/kg was found in 5 dogs. The time at which the contrast medium appeared or disappeared in the lymph node or lymphatic vessels varied at each dose of contrast medium (Tables 1, 2).

In terms of image quality score, there were statistically significant differences between 30 mg/kg and 60 mg/kg and between 30 mg/kg and the 90 mg/kg; however, there was no
significant difference between 60 mg/lkg and 90 mg/lkg (Table 3). Therefore, radiographic lymphography with doses of 60 mg/lkg and 90 mg/lkg was determined to be of a higher diagnostic quality based on scores of 3 or higher in the blind testing. A detailed evaluation of agreement among the three observers in relation to diagnostic image quality was performed by means of kappa statistics. Overall, strong agreement among the test participants was noted (Table 4).

The thoracic ducts of all dogs were clearly observed in computed tomographic images taken at the time of scanning of the thoracic ducts (Fig. 3). In all subject dogs, the thoracic ducts were slightly right of midline between the right lung and azygos vein and just anterior to the spine at the level of T11 in the caudal thorax. At the level of the aortic arch, the thoracic duct crosses to the left side of the body at aorta ventral T6. In the thoracic inlet, the thoracic duct coursed anteriad between the esophagus medially and the left subclavian artery laterally.

The number of thoracic ducts observed by computed tomographic imaging varied from one to four depending on the location. However, there were no significant differences in the number of thoracic ducts between computed tomography and radiography at each anatomic location (Table 5).

DISCUSSION

The popliteal lymph node is palpated more easily in lymph nodes located in the hind legs [9]. This location means that problems can be easily remedied with fingers during palpation, and this lymph node is suitable for admin-
The administration of contrast medium or fine needle aspiration. The results of the study reported here indicate that detailed delineation of the thoracic duct and its branches and lymphaticovenous anastomoses was possible via an injection of contrast medium directly into a popliteal lymph node in all the dogs.

Overall, radiographic evaluations involving direct injection of contrast medium into a popliteal lymph node successfully delineated the popliteal lymph node, inguinal lymph node, external iliac lymph node, lumbar lymph vessels, cisterna chyli, thoracic duct and its branches as far cranial as the thoracic inlet and lymphaticovenous anastomoses in the dogs. The radiographic details of these anatomic features obtained with this procedure were similar to those obtained with lymphatic catheterization.

This method does not require further surgical treatment; only checks are required by ultrasound to see whether the contrast medium is accurately injected into a popliteal lymph node. Often, the contrast medium is not injected in a popliteal lymph node but instead leaks out to form a cyst. In this case, an ultrasound can be used to locate the needle in the popliteal lymph node and inject the contrast medium.

<table>
<thead>
<tr>
<th>Location</th>
<th>Dose of Contrast Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 mgI/kg</td>
</tr>
<tr>
<td></td>
<td>60 mgI/kg</td>
</tr>
<tr>
<td></td>
<td>90 mgI/kg</td>
</tr>
<tr>
<td>Popliteal LN</td>
<td>497.2 ± 96.6</td>
</tr>
<tr>
<td></td>
<td>741.0 ± 184.3</td>
</tr>
<tr>
<td></td>
<td>812.2 ± 70.9</td>
</tr>
<tr>
<td>Inguinal LN</td>
<td>521.6 ± 65.8</td>
</tr>
<tr>
<td></td>
<td>746.6 ± 180.8</td>
</tr>
<tr>
<td></td>
<td>748.8 ± 67.8</td>
</tr>
<tr>
<td>External iliac LN</td>
<td>556.8 ± 13.2</td>
</tr>
<tr>
<td></td>
<td>703.0 ± 144.5</td>
</tr>
<tr>
<td></td>
<td>767.0 ± 68.9</td>
</tr>
<tr>
<td>Lumbar LV</td>
<td>377.4 ± 62.8</td>
</tr>
<tr>
<td></td>
<td>398.8 ± 54.2</td>
</tr>
<tr>
<td></td>
<td>667.4 ± 97.7</td>
</tr>
<tr>
<td>Cisterna chyli</td>
<td>659.0 ± 151.1</td>
</tr>
<tr>
<td></td>
<td>700.6 ± 254.0</td>
</tr>
<tr>
<td></td>
<td>996.8 ± 30.9</td>
</tr>
<tr>
<td>Thoracic duct</td>
<td>603.8 ± 147.5</td>
</tr>
<tr>
<td></td>
<td>830.6 ± 286.2</td>
</tr>
<tr>
<td></td>
<td>1024.2 ± 84.5</td>
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</table>

Table 3. Image Quality Scores for Lymphography Among the Three Doses at the First and Second Examinations (n=5)

<table>
<thead>
<tr>
<th>Observer No.</th>
<th>30 mgI/kg**</th>
<th>60 mgI/kg*</th>
<th>90 mgI/kg†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st Exam</td>
<td>2nd Exam</td>
<td>1st Exam</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2.4</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>1.6</td>
<td>1.8</td>
<td>3.2</td>
</tr>
<tr>
<td>3</td>
<td>1.8</td>
<td>2</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Table 4. Interobserver and Intraobserver Reliability for Popliteal Lymphography

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reliability Coefficient (κ)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraobserver</td>
<td>Observer 1</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>Observer 2</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>Observer 3</td>
<td>0.77</td>
</tr>
<tr>
<td>Interobserver</td>
<td>Observer 1 vs observer 2</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>Observer 2 vs observer 3</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>Observer 1 vs observer 3</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Fig. 2. Lateral radiographic views of the thorax after injection of three different doses of a contrast medium: (A, B and C: 30, 60 and 90 mgI/kg, respectively) into a popliteal lymph node. The thoracic duct and branches were visible with all doses. The thoracic ducts were more clearly visible depending on increasing dosage of contrast medium.
again. However, caution is required as an ultrasonographic image will not be obtainable if an air bubble is present within the contrast medium and is inadvertently injected into the lymph node. Brisson et al. stated that if the mesenteric lymph node is moved during a laparoscopy procedure, then the contrast medium could leak around the injection point [3]. In our study, the contrast medium injected into the popliteal lymph node could be checked accurately with an ultrasound. This study outlines an easier and less invasive method than the recent study reported by Brisson et al. [1].

Our experiment was carried out after anesthesizing the animals; however, since it did not require surgical treatment, it may be possible to perform lymphography by injecting the contrast medium into a popliteal lymph node without anesthesia.

Naganobu et al. described performance of popliteal lymphography by injection of iohexol with a volume of 5 to 10 mL and an injection time of an average of 5 min. Although we used similar methods, smaller amounts of iohexol (60 mgI/kg) were used, which facilitates a much faster injection time without a loss of image quality in lymphatic duct opacification [8].

A previous study reported that radiography with an inadequate delay time requires additional injection of iohexol into the popliteal lymph node [8]. The present study also suggests that determination of the delay time for computed tomographic scanning is necessary due to the disappearing nature of the contrast media in the thoracic duct. In the present study, the mean filling and duration time were acquired through fluoroscopic observation (Tables 1, 2), and scanning conditions comprised of a delay time of $118 \pm 10.4$ seconds after injection of iohexol into the popliteal lymph node within $830 \pm 28.2$ seconds was applied to the computed tomographic study; this enabled acquisition of excellent image quality for the computed tomographic images in all 5 dogs without inadequate opacification of the thoracic duct.

It was determined that the proper dose of contrast medium was 60 mgI/kg for our study because both 60 mgI/kg and 90 mgI/kg provide good images for identification of the thoracic duct. However, the contrast medium leaked around the popliteal lymph node at 90 mgI/kg in all subject dogs after injection.

The lymphatic vessels collect excess interstitial fluid from tissues throughout the body and carry it to the subclavian veins, where the lymph re-enters the bloodstream. Lymph moves through lymph vessels via bulk flow. The driving force of this flow is the interstitial fluid hydrostatic pressure minus the subclavian vein pressure. Lymph flow is also promoted by the massaging action exerted on lymph vessels by contraction and relaxation of skeletal muscles. The lymph vessels contain one-way valves, which prevent the backflow of lymph. Thus, the massaging action of skeletal muscle propels lymph in one direction only, toward the subclavian [2, 13]. The time at which the inguinal lymph node, external iliac lymph node, cisterna chyli and thoracic duct were observed after injection of contrast medium into the popliteal lymph node showed a consistent pattern regardless of the dose of contrast medium. In our experiment, there was no difference in time depending on the dose, and it is assumed that the quantity of the interstitial fluid did not change much since it was small even when there was a difference in the amount of contrast medium.

Previous studies have suggested that the use of computed tomography in lymphangiography could be used to check thoracic ducts without superimposition and thus obtain more accurate information. Our experiment also checked the thoracic ducts using computed tomography. We estab-

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### Table 5. Comparison of the Number of Thoracic Ducts between Radiographic and Computed Tomographic Images after Injection of Contrast Medium (60 mgI/kg)

<table>
<thead>
<tr>
<th>Vertebra</th>
<th>Type of Image</th>
<th>Radiography</th>
<th>CT</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>1.0 ± 0.0</td>
<td>1.0 ± 0.0</td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>T12</td>
<td>1.2 ± 0.4</td>
<td>1.4 ± 0.5</td>
<td></td>
<td>0.083</td>
</tr>
<tr>
<td>T10</td>
<td>1.7 ± 0.7</td>
<td>2.4 ± 1.0</td>
<td></td>
<td>0.102</td>
</tr>
<tr>
<td>T8</td>
<td>2.1 ± 0.6</td>
<td>2.2 ± 0.7</td>
<td></td>
<td>0.157</td>
</tr>
<tr>
<td>T6</td>
<td>1.6 ± 0.5</td>
<td>1.7 ± 0.7</td>
<td></td>
<td>0.157</td>
</tr>
</tbody>
</table>

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**Fig. 3.** Transverse computed tomographic images of the thorax after injection of contrast medium (60 mgI/kg) into a popliteal lymph node with the subject dog in dorsal recumbency. Note the two branches of the thoracic duct at T10 level (A: white arrow) and the convergence of branches at T8 level (B: white arrow).
lished the time delay required for identification of the thoracic duct after injection of a contrast medium into the popliteal lymph node. When performing computed tomography at the established time, we could identify thoracic ducts in all 5 subject dogs. In a previous study, Esteline concluded that computed tomography allows users to identify more thoracic duct branches than is possible with radiographic lymphangiography [4]. Unlike previous studies, our study revealed that there was no significant difference between CT and radiographic image in terms of the number of thoracic ducts in any region. This may be due to the small population of dogs used because more thoracic ducts were counted with computed tomographic images at every anatomical level, although there was no statistically significant difference. However, patients do not need to be repositioned, images can be manipulated to better observe the target tissue, image contrast is vastly enhanced and small differences in contrast can be detected during computed tomographic scanning [3]. Lymphography using computed tomography for identification of the thoracic duct would be useful for ligation of the thoracic duct [4, 12]. Therefore, although the number of thoracic ducts detected by computed tomography and conventional radiography did not significantly differ, it is assumed that computed tomography is the superior modality for delineation of the thoracic duct after popliteal lymphography.

Surgical options in animals with chylothorax include ligation of the ruptured thoracic ducts [10]. The advantage of thoracic duct ligation is that it results in complete resolution of pleural fluid. Disadvantages include a long operative time and the possible difficulty of performing mesenteric lymphangiography [5]. Numerous researchers have studied the methods of checking and ligating thoracic ducts as components of non-invasive methods. Hitherto, the usual practice has been to inject a contrast medium into the mesenteric lymph node or mesenteric lymph vessel by laparotomy, which requires an extended amount of time under anesthesia and surgical treatment [1]. Recently, some surgeons have performed thoracic duct ligation using thoracoscopy [5]. Since our study showed that injection of a contrast medium into a popliteal lymph node successfully delineated the thoracic duct without surgical treatment, it is likely that popliteal lymphangiography combined with thoracoscopic thoracic duct ligation improves surgical outcome and speeds up postoperative recovery [14].

Therefore, computed tomographic scanning of the thorax with a delay time of 118 ± 10.4 seconds after injection of 60 mgI/kg of iohexol into a popliteal lymph node within 830 ± 28.2 seconds may be useful in demonstrating the nature of lymphatic damage, assisting in selection of patients for surgery and helping determine the type of surgery to be performed.

REFERENCES