

Visualisation of the thoracic duct after popliteal lymph node injection in the pig: comparison of radiographic and thoracoscopic techniques

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ABSTRACT: Radiographic contrast studies have been recommended to identify the thoracic duct (TD) and its branches before and after surgery for total occlusion. The macroscopic identification of the TD and its branches during surgery usually involves injection of methylene blue (MB). Radiographic contrast and methylene blue can be injected into different anatomical structures (lymph node, lymph vessel, *s.c.*). The purpose of this study was to compare two different techniques (radiographic and thoracoscopic) for visualisation of the TD after intrapopliteal lymph node injection in the pig. Six piglets from the same litter (two males and four females), two months of age were used. Iohexol at 245 mg/ml was injected into the left popliteal lymph node (LN) under general anaesthesia; hindlimb, abdominal, and thoracic radiographs were taken. A 0.25% methylene blue solution was injected into the right popliteal lymph node and the thoracic duct colouration was assessed thoracoscopically. The thoracic duct was visualised radiographically in one out of six pigs after iohexol injection and thoracoscopically in five out of six pigs after methylene blue injection. The difference was statistically significant ($P = 0.040$). Popliteal LN lymphangiography using iohexol at 245 mg/ml in piglets should not be the recommended method for TD visualisation. Intrapopliteal injection of a 0.25% solution of methylene blue is recommended as a method of TD visualisation prior to thoracic duct ligation.

Keywords: iohexol; methylene blue; pig; popliteal lymph node; thoracic duct

List of abbreviations

TD = thoracic duct, LN = lymph node, MB = methylene blue

Chylothorax is characterised by the abnormal accumulation of chyle in the thoracic cavity. Because the observed resolution rates with conservative management are low, surgery is usually preferred. Current surgical management of chylothorax consists of the following: TD ligation (Fossum et al. 1986; Fossum et al. 1991); partial pericardiectomy with or without TD ligation (Fossum et al. 2004; Carobbi et al. 2008); clipping of the TD during thoracoscopy (Radlinsky et al. 2002); clipping or sealing of the TD and pericardiectomy during thoracoscopy (Allman et al. 2010; Haimel et al. 2012); cisterna chyli ablation with or without TD ligation (Hayashi et al. 2005; Sakals et al. 2011); omentalisation with or without TD ligation (Williams and Niles 1999; Talavera et al. 2009; Stewart and Padgett 2010); or pleurodesis (Radlinsky 2012).

Radiographic contrast studies have been recommended (1) to identify the thoracic duct and its tributaries before surgery and (2) to confirm total occlusion of the TD and its branches after surgery. On the other hand, macroscopic identification of the TD and its branches during surgery, via either thoracotomy or thoracoscopy, usually involves injection of MB. Since pigs are often used as experimental models for the assessment of surgical technique efficacy (Novitsky et al. 2005; Ashitate et al. 2011), in this study, the best method for identifying the TD in pigs was evaluated. Many surgeons recommend TD lymphangiography before and/or after TD ligation, clipping or sealing to confirm cessation of lymph flow. To this end, the TD and its branches can be visualised after radiopaque contrast media or dye injection (MB) into different

anatomical locations and subsequent radiographic control or thoracotomy/thoracoscopy with differing specificity in humans and animals.

The recommended technique, which is strongly preferred, for minimising morbidity from the surgical management of chylothorax is the injection of contrast media or MB in the popliteal LN. This technique does not require any laparotomy or the popliteal LN to be lodged subcutaneously; further, popliteal LN detection and injection are technically simple.

We are unaware of any previous studies comparing the efficacy of iohexol contrast media with MB dye, which are used for the identification of TD by injection into the popliteal LN in pigs. The objective of this study was to assess the efficacy of MB and contrast media iohexol injection into the popliteal LN for the visualisation of TD in pigs.

MATERIAL AND METHODS

Animals. The experimental protocol was approved by the Department of Veterinary Science and was conducted in accordance with the guidelines for the treatment of laboratory animals. Emotional reassurance (gentle restraint, petting, and talking) was provided by the handler. Six piglets (two males and four females) at two months of age from the same litter were used.

Pre-op preparation. Food was withheld for 12 h and water for 2 h before the experiment began. Each piglet was anaesthetised with intramuscular injection of xylazine (2 mg/kg) (Xylapan, Vetoquinol, France) and ketamine (10 mg/kg) (Narketan, Vetoquinol, France). An intravenous catheter was placed in a cephalic vein, and 5–10 mg/kg of thiopental sodium (Thiopental, Nycomed, Norway) was administered. The trachea was intubated with a 5.5–6.5 *i.d.* endotracheal tube. A temporary tracheotomy was performed if the endotracheal intubation was unsuccessful after five attempts. Each patient was clipped and aseptically prepared on both caudal thighs and on the right hemithorax just before skin incision. Plain thoracic radiographs (left to right and dorsoventral) were performed to rule out any underlying thoracic pathology.

A 4 to 5 cm skin incision was performed in the left popliteal region. The left popliteal LN was located and dissected. A curved haemostat or ligature was attached at the ventral pole of the popliteal LN in order to facilitate its traction during subsequent manipulation. A 70% solution (7 ml of iohexol and

3 ml of saline) of iohexol at 350 mg/ml (Omnipaque 350, GE Healthcare Inc., UK) was prepared. With the patient in right lateral recumbency, 5 ml of the prepared solution was injected directly into the left popliteal LN using a 21G butterfly catheter. Right lateral and ventro-dorsal thoracic radiographs were taken 3 min after the injection. Ten minutes after the first injection, another 5 ml of the prepared solution was injected, and the radiographs were repeated. The adequacy of the intra-popliteal injection was evaluated by a radiograph of the left hindlimb, pelvic area, and caudal abdomen 3 min and 10 min after the last thoracic radiograph. The iohexol lymphangiography was evaluated by two surgeons and one radiologist who graded it as positive when the dye was seen in the thoracic duct. If the lymphangiography did not reach the thoracic duct, its maximal proximal extension was noted. After completion of the radiological study, the patient was moved to the operating theatre and anaesthesia was maintained using isoflurane combined with oxygen delivered through a circle rebreathing system. Lactated Ringer's solution was administered intravenously at 10 ml/kg/h. Pain was controlled using fentanyl citrate (Fentanyl, Janssen-Cilag, Switzerland) at a dose of 3 µg/kg intravenously followed by 0.1–0.2 µg/kg/min CRI. Heart rate, respiration rate, body temperature, ECG, SaO₂, arterial blood pressure, and EtCO₂ were monitored (S/5™ Light, DatexOhmeda, Madison, WI). Positive pressure ventilation at a tidal volume of 5–10 ml/kg and 12–20 breaths per minute (Aespire 7900, DatexOhmeda, Madison, WI) was used.

Ten millilitres of a 0.25% MB solution were prepared by dilution of the original solution (Patentblau V25 mg/ml, Guerbet, France) with saline at a 1 : 9 ratio. With the animals in left lateral recumbency, the right popliteal LN was dissected using the same technique described above. The animals were then positioned in sternal recumbency. A cannula with a 6 mm diameter, 85 mm length, and multifunctional valve (62160 WZ, Storz, Switzerland) and two 6 mm Endotip cannulas (60160 MTR, Ternamian Endo TIP™ Cannula, Storz, Switzerland) were inserted at the dorsal third of the right hemithorax along a curve from the 8th to the 11th intercostal space. Intra-thoracic CO₂ insufflation at a maximum pressure of 5 mm Hg was used to improve the intra-thoracic view as needed and interrupted at the anaesthetist's request. A 5 mm, zero degree, 29 cm long laparoscope (62046 AA, Storz Hopkins II, Switzerland) was selected, and a

5 mm endoscopic palpation probe (62175 T, Storz, Switzerland) and 5 mm, 36 cm long grasping forceps (66323 BA, Clickline BABCOCK Grasping forceps, Storz, Switzerland) were respectively used to push the diaphragm caudally and the diaphragmatic lung lobe ventrally. The scope was directed towards the caudo-dorsal border of the right hemithorax viewing the region between the costo-diaphragmatic angle caudally, the ventral wall of the aorta ventrally, the sympathetic trunk dorsally and the 6th costo-vertebral junction cranially. The MB solution was injected into the right popliteal LN using a 21G butterfly catheter. The injection proceeded until an LN enlargement was observed and resistance was felt. The process was stopped for one minute and then repeated up to four times. If the TD was not visible after four intrapopliteal injections, administration of MB was terminated, and TD visualisation in the pig was classified as unsuccessful. When TD colouration was observed, MB administration was continued only until the LN was enlarged. Both operators and one independent observer (anaesthetist) examined the thoracoscopic image on the screen while the MB solution was injected. The following variables were evaluated:

The amount of time between the MB injection and the first TD staining at the level of the costo-diaphragmatic angle.

The staining grade, one minute after the first colouration appeared, using the scale developed by Enwiller et al. (2003): grade 0 = normal appearing tissue; grade 1 = faint blue or equivocal staining; grade 2 = dusky slate blue, obvious staining; grade 3 = intense blue staining.

The entire thoracoscopic procedure was recorded (AIDA compact, Storz, Switzerland). At the end of the procedure, the animals were euthanised by intravenous administration of T-61 (Intervet). A necropsy was then performed and the path of accumulation of MB was followed from the popliteal LN to the TD.

Statistical analyse. The success rates of TD visualisation with iohexol and thoracic radiographs or MB and thoracoscopy were analyzed and compared using a nonparametric Chi square test (2×2 table, Fischer exact P) in Statistica 10 software. $P < 0.05$ was considered significant.

RESULTS

The mean body weight of the piglets was 16.3 ± 1.8 kg. Thoracic radiographs performed before

contrast media injection did not show evidence of any underlying thoracic disease. In two of the six animals, a temporary tracheotomy was performed. No life-threatening complications or significant changes were observed during the monitoring of the patients under anaesthesia. Hypoxemia, defined as $SpO_2 < 93\%$, was not observed in any pig at any point while using CO_2 insufflation of the thoracic cavity at a maximum of 5 mm Hg pressure.

Leakage of either iohexol or MB occurred in 10 out of 12 LN administrations. The thoracic duct lymphangiography using iohexol was observed radiographically in only one pig (16.6%) on one thoracic radiograph performed 3 min after the injection (Figure 1). The iohexol lymphangiography was not interpreted as positive in any of the other X-rays. The pelvic limb radiographs, performed as a control 10 min after the injection, confirmed the intrapopliteal injection of contrast medium in all animals and a slow proximal diffusion of contrast media to the LN of the iliosacral lymph centre in 50% (3/6) of the animals (Figure 2). No diffusion of contrast media through the lymph vessels was observed beyond the popliteal LN in the remaining 50% (3/6) of the animals.

Thoracic duct colouration using MB was observed thoracoscopically in five out of six pigs (Figure 3). The mean length of time from the intra-popliteal injection to the thoracoscopic visualisation in five pigs was 153.8 ± 106.3 s (range 42–300 s). In one pig the injection of MB contained an average of 3–4 ml



Figure 1. Right lateral thoracic radiograph of patient No. IV performed 3 min after injection of iohexol into the popliteal lymph node. The path (yellow arrows) of the thoracic duct is readily visible in the entire thoracic cavity



Figure 2. Right lateral abdominal and pelvic limbs radiographs performed 10 min after the injection. This X-ray confirms the adequate intra-popliteal injection of the contrast medium (arrow) and the slow proximal diffusion of contrast media to the iliosacral lymph centre (arrowhead)

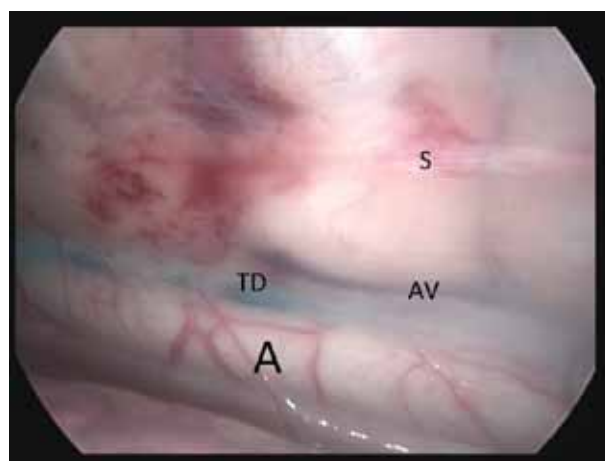


Figure 3. Thoracoscopic view of the caudo-dorsal right hemi-thorax. The coloured thoracic duct (TD) is readily visible between the descending aorta (A), right azygos vein (AV) and sympathetic trunk (S)

of MB and LN enlargement took close to 30 s. More than 10 ml of MB solution (12 ml) was administered in only one pig. In this pig, colouration of TD was not observed, and the necropsy revealed that the MB had reached the level of the right kidney. TD colouration was observed in three pigs after the first MB injection and in two pigs after the second MB injection. In five pigs (in which TD colouration was observed), the mean staining intensity was graded as 2.40 ± 0.42 (range 2–3) one minute after the beginning of TD colouration.

The difference between the number of animals in which visualisation of TD was observed after injection of iohexol and radiography (1/6) or MB and thoracoscopy (5/6) was statistically significant ($P = 0.040$).

DISCUSSION

Several different colouration methods, vital dyes and injection sites have been tested in different animal species in the literature. In the dog, MB injection resulted in a positive thoracic duct visualisation in 10/10 patients after intramesenteric LN injection versus 6/10 after intra-popliteal visualisation (Enwiller et al. 2003). Thoracic duct colouration via popliteal LN injection (MB) failed in one dog (1/3) (Allman et al. 2010). Leasure et al. (2011) cannulated an efferent mesenteric lymphatic vessel in the region of the ileoceccocolic LNs in dogs;

after MB injection, the TD colouration was successful in all dogs (5/5). Sakals et al. (2011) achieved a cisterna chyli colouration after intrapopliteal MB injection in 12/14 adult dogs. In one dog, in which colouration was not observed, MB was observed in the abdominal lymphatics, but the cisterna chyli was not found.

In pigs, Novitsky et al. (2005) injected MB in tissues around the lesser gastric curve and observed a positive TD colouration in all cases (4/4). Ashitate et al. (2011) observed that MB injection into an inguinal LN resulted in successful TD visualisation in all pigs (8/8). When India ink or MB was injected in the testicular parenchyma of rabbits, TD was visualised in 3/3 patients (Tsuruno et al. 2009).

In our study, despite the small number of patients (in accordance with 3R's rule-reduction of animals) a significant difference in TD visualisation was observed between the method of iohexol injection at a concentration of 245 mg/ml followed by thoracic X-rays and MB injection followed by thoracoscopic examination. Thus, colouration of the TD was successfully achieved by MB injection and visualised thoracoscopically in five out of six animals. In the sixth pig, where the colouration was unsuccessful, the necropsy revealed that the MB had only reached the level of the right kidney. In that same pig, the tissue surrounding the popliteal LN was heavily coloured, suggesting that the LN capsule ruptured during injection and that MB was actually absorbed from the surrounding tissues.

Nevertheless, in our study, the success rate of TD visualisation after MB injection was higher than in Enwiller's study on dogs. Since Allman et al. (2010) were unsuccessful in their attempts to inject MB percutaneously into the popliteal LN in dogs, we opted for the dissection and direct visualisation of LN in our study. The dissection of the popliteal region was a simple procedure and did not require the usage of magnification.

In this study, thoracic duct lymphangiography using intra-popliteal injection of iohexol at 245 mg/ml was successful in only one out of six pigs. In comparison, results of previous studies have been variable. For instance, Naganobu et al. (2006) observed a 100% contrast uptake into the TD after an injection of 10 ml of iohexol at 300 mg/ml into the popliteal LN of healthy experimental dogs. These results suggest that the success rate of popliteal lymphangiography in dogs is higher than in pigs. Allman et al. (2010) injected meglumine diatrizoate into a mesenteric lymphatic vessel and obtained a positive TD lymphangiographic study in only one out of three dogs, whereas Leasure et al. (2011) visualised the TD in six out of six dogs after injection of meglumine and sodium diatrizoate into a mesenteric lymphatic vessel. Tsuruno et al. (2009) injected iohexol into the testicles of rabbits. Visualisation of the TD was obvious in five out of nine patients (those where 240 mg/ml iohexol was used at 37°C). Taken together, in cases where cannulation of an abdominal lymph vessel was used, the success rate with contrast media or with MB seems to be higher regardless of the animal species (Radlinsky et al. 2002; Fossum et al. 2004; Leasure et al. 2011). However, since the catheterisation of an abdominal lymph vessel is a demanding procedure that requires an open approach to the abdominal cavity, our study aimed to evaluate alternative techniques.

Several explanations can be proposed for the statistically significant difference in success rates between MB and iohexol injection.

First, the molecular weight of iohexol is 821 g/mol, and the molecular weight of MB is 320 g/mol. Also, the dynamic viscosity of iohexol-350 at 37 °C is 10.4 cP, whereas that of the aqueous solution of MB is < 1 cP. It is thought that high-viscosity products have difficulties flowing through narrow lymph vessels. Tsuruno et al. (2009) concluded that iohexol-240 is an optimal agent for TD visualisation in rabbits after intratesticular injection. As iohexol-240 is unregistered in our country, we used iohexol-350 (diluted in saline) to obtain a 245 mg/ml

solution. It can be hypothesised that dilution actually reduces the viscosity and should have improved the flow. Also, warming the contrast media to a temperature of 37 °C improved the flow rate of contrast medium after intratesticular injection in rabbits, as increasing the temperature of a fluid reduces its viscosity (Tsuruno et al. 2009). In our study, iohexol was injected at room temperature (26 °C); we suspect that better results would have been obtained after warming the contrast agent.

It does not seem that leakage during injection affected our results. During the injection of iohexol or MB in the LN, leakage was observed in 5/6 pigs. In the study of Enwiller et al. (2003), the MB solution leaked from the LN injection site in all dogs. Leakage can be the consequence of tissue damage during dissection, inadvertent perforation of the capsule with the tip of the butterfly catheter, or capsule rupture because of administration of a high volume within a short time period. Therefore, it is recommended: (1) to carefully dissect the LN, (2) to not perforate the capsule during the puncture with the butterfly catheter, and (3) to inject solutions slowly or to use a syringe pump. During this study, we observed that a lack of resistance during manual injection was a sign of capsule perforation.

Thoracoscopic visualisation of the TD has been documented in dogs (Radlinsky et al. 2002; Allman et al. 2010; Leasure et al. 2011) and in pigs (Ashitate et al. 2011). Positioning the patient in sternal recumbency allows better access to the caudal mediastinal structures and to the tissues located from the dorsal to the aorta. In addition, in our study, a slight pneumothorax with a maximal pressure of 5 mm Hg was created, and a palpation probe was used to move away the dorsal border of the diaphragm and the caudal lung lobe; the visualisation of structures in the right caudodorsal thorax was always excellent. Wolfer et al. (1994) have used thoracic insufflation during thoracoscopy in pigs and concluded that low-pressure insufflation (< 10 mm Hg) is a safe adjunct to the conduct of routine thoracoscopic surgical procedures. Since the TD was always located in the same position and no accessory branches were identified after MB injection, one might question if colouration of the TD is necessary. Further studies are needed to confirm that ligation or sealing can be successfully performed without colouration.

There are several limitations to this study:

First, the injection side (left for iohexol and right for methylene blue) was not randomised. However,

as Singh et al. (2010) did not find a significant difference in the number of observed lymphatic branches after left or right intrapopliteal iohexol injection during a radiographic and CT scan study in the dog, we hypothesised that the injection side should not have any impact on the results.

Second, there were some differences in the two protocols. First, the animals breathed spontaneously during iohexol injection and were mechanically ventilated during MB injection. Also, CO₂ was insufflated into the chest (with a maximum pressure of 5 mm Hg) as needed during the thoracoscopic examination. Both positive pressure ventilation and intrathoracic insufflation increase the intrathoracic pressure and should have negatively affected the MB flow through the TD. Lattuada and Hedenstiema (2006) also concluded that spontaneous breathing increases lymph flow in pigs in comparison to mechanically-ventilated pigs.

Comparing the protocols further, patients were in right lateral recumbency during iohexol injection and in sternal recumbency during the MB injection. The sternal recumbency used during MB injection may have elevated the intra-abdominal pressure and further compromised the intra-abdominal lymph flow. Also, we hypothesised that the previous injection of iohexol into the left popliteal lymph node should not have further influenced the lymphatic flow, because the interval between the two injections was more than 30 min. As lymphatic flow in pigs has been estimated to be between 2.5 and 6.9 ml/min (Lattuada and Hedenstiema 2006) iohexol should have disappeared from the lymph vessels at the time of MB injection.

Taken together, these protocol differences should have negatively influenced the flow of MB in the TD. Despite this, thoracoscopic TD visualisation after intra-popliteal MB injection was significantly superior to that obtained after lymphangiography with iohexol.

A further caveat to our study is that since the piglets were only two months old, the diameter of the lymph vessels was small, and combined with the high viscosity of iohexol, may have contributed to a lower success rate of TD colouration.

Finally, the number of patients was limited and further studies on clinical, adult patients are warranted.

When a simple method of TD visualisation is necessary for surgical or experimental purposes in pigs, intra-popliteal injection of MB followed by thoracoscopy can be recommended. Based on our results, popliteal LN lymphangiography using

iohexol at 245 mg/ml used at room temperature in pigs should not be the recommended method for TD visualisation.

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