Variation and Visualization of the Lymphatic Drainage Routes in Normal Canine Mammary Glands.

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Abstract

The lymphatic drainage pattern of normal canine mammary glands was investigated in an anatomical study using India ink, and the usefulness of three-dimensional computed tomographic analysis (3D-CT) with contrast media for visualization of sentinel lymph nodes was examined in a clinical setting.

In the anatomical study, the first and second mammary glands consistently drained into the ipsilateral axillary lymph nodes in all dogs. In two of three cases, the third mammary gland drained simultaneously into the ipsilateral axillary lymph nodes cranially and into the superficial inguinal lymph nodes caudally. In one of these two cases, the lymph drained into the contralateral inguinal lymph nodes via the pelvic lymphatic plexus. In the remaining case, the third mammary gland only drained caudally into the ipsilateral superficial inguinal lymph nodes. In five cases, the fourth mammary gland, and in two cases, the fifth mammary gland, usually drained into the ipsilateral superficial inguinal lymph nodes and medial iliac lymph nodes in the abdominal cavity. However, in one case, the fourth mammary gland drained into the contralateral inguinal lymph nodes via the pelvic lymphatic plexus, as also seen in the third mammary gland.

The CT analysis showed that the lymphatic pathways draining directly from the injection sites of small doses of contrast medium were quickly and sufficiently enhanced in the animals without any significant late adverse effects. In particular, topographic 3D-CT lymphography provided detailed anatomical information on individual lymphatic channels and nodes without overlaps with the surrounding structures. Thus, visualization of lymph drainage using simple, noninvasive 3D-CT is useful for easy identification of the lymph drainage route and is necessary not only to determine the most appropriate surgical treatment of mammary tumors, but also to determine an accurate postsurgical prognosis.

Key word: Anatomical study, Dog, Lymph Drainage Route, Mammary Glands, 3D-CT

Introduction

The lymphatic system is considered the main route for mammary tumor metastases, and surgery for a mammary tumor implies removal of the tumor along with the gland-associated lymph nodes [5]. Although there is a fairly standard lym-
phatic drainage pattern and route from the canine mammary glands [1, 6-8, 11, 12], there are also variations in drainage among individuals. In addition, mammary neoplasia is known to sometimes alter the lymphatic drainage pattern, forming new draining channels and recruiting a large number of lymph nodes [2, 8, 13]. Consequently, visualization of the lymphatic drainage route from each mammary gland for each individual should be of high importance for the surgeon, not only for determining the most appropriate surgical excision but also to provide an accurate postsurgical prognosis.

The purpose of the present study was to investigate the variations in the lymphatic drainage pattern of canine mammary glands using an anatomical technique and to examine the usefulness of computed tomography (CT) lymphography to visualize the sentinel lymph nodes for clinical use.

**Materials and Methods**

(1) Preparation of anatomical specimens by administration of India ink to confirm lymph vessel anatomy

Eighteen clinically healthy intact female beagles (body weight range, 8.3 to 12.7 kg; age range, 1.5 to 3.0 years old) previously employed for educational purposes at Veterinary Surgery Laboratory, Azabu University, were used in this experiment. After the laboratory practice, the dogs were euthanized by intravenous administration of a lethal dose of pentobarbital. Immediately following euthanasia, warm India ink dissolved in physiological saline at a dose of 0.2 ml/kg body weight was injected into the right side first or cranial thoracic mammary gland (four cases, A-D), the second or caudal thoracic mammary gland (four cases, E-H), the third or cranial abdominal mammary gland (three cases, I-K), the fourth or caudal abdominal mammary gland (five cases, L-P), or the fifth inguinal mammary gland (two cases, Q and R). The dogs then underwent whole-body retrograde fixation with a mixture of ethylene glycol, liquefied phenol, formalin, and methanol mixed in the ratio of 24:8:1:16. After complete fixation, the absorption fields and pathways of the drainage routes were observed macroscopically and by stereoscopic microscopy.

(2) CT analysis of lymphographic images of the lymphatic system after injection of angiographic contrast medium

Three healthy intact female beagles (body weights of Dogs 1, 2 and 3 were 10.8, 9.35 and 9.55 kg, and they were, respectively, 2.8, 3.3 and 3.5 years old) were used for CT imaging. The animals were treated in accordance with the guidelines of the Office of Laboratory Animal Welfare at Azabu University. Atropine sulfate (0.05 mg/kg sc) and butorphanol tartrate (0.1 mg/kg sc) were used as anesthetic premedication and propofol (6–7 mg/kg iv) were administered to induce anesthesia. The dogs were intubated and were maintained with inhalation of isoflurane in 100% oxygen. Each anesthetized animal was placed in the supine position for CT imaging.

For CT lymphography, using a 24-G needle and at a dose of 0.2 ml/kg, two warmed water-soluble angiographic contrast media (iopamidol: Iopamiron-370, high consistency medium; and Iopamiron-150, low consistency medium: Nippon Schering, Osaka, Japan) were injected into the subcutaneous portion near/under the teat of the mammary gland. In Dog 1, Iopamiron-370 was injected into the first through fifth mammary glands on both sides. In Dog 2, Iopamiron-370 was injected into the right second and third mammary glands, and left first and second mammary glands. In Dog 3, Iopamiron-150 was injected into each subcutaneous portion of the right second and left fourth mammary glands. In each experiment, we confirmed that the injected contrast medium did not remain in the body.

After injection of the contrast medium, the injected area was gently massaged for 5 min to facilitate absorption of the contrast medium by the intramammary lymphatics. CT imaging was carried out 1, 5, 10, 15, and 30 min after massage. The 3D reconstruction of the acquired image data (DICOM data), reading the images in DICOM files and export of TIFF files were performed using Osirix version 2.7.5 (Osirix Foundation, Geneva, Switzerland). The CT scanner used in this experiment was a Toshiba Asteion TSX-021B single-slice device, and imaging conditions were as follows: 120 kV; 60–70 mA; helical pitch, 1.5; scan speed, 0.75 s; slice thickness, 2 mm; and reconstruction algorithm, FC80. All procedures in this study were in accordance with the guidelines approved by the Animal Research Committee of Azabu University.
Results

(1) Dissection after India ink administration

Anatomical observations after administration of India ink are shown in Table 1. Analysis of the anatomical specimens revealed that all the thoracic mammary glands (first and second or cranial and caudal thoracic mammary glands) of eight cases (A–H) consistently drained into the ipsilateral axillary lymph nodes. In the third or cranial abdominal mammary gland of three cases (I, J and K), two cases (I and J) drained simultaneously into the ipsilateral axillary lymph nodes cranially and into the superficial inguinal lymph nodes caudally. Moreover, in one (I) of the two cases, the lymph drained into the contralateral inguinal lymph nodes via the pelvic lymphatic plexus. In the last of those three cases, K, the third mammary gland only drained caudally into the ipsilateral superficial inguinal lymph nodes. The fourth or caudal abdominal mammary gland of five cases (L–P) and the fifth or inguinal mammary gland of two cases (Q and R) usually drained into the ipsilateral superficial inguinal lymph nodes and medial iliac lymph nodes. However, in case L the lymph drainage path from the fourth mammary gland was extended downward to the contralateral inguinal lymph nodes via the ipsilateral superficial inguinal lymph node and pelvic lymphatic plexus, as also seen in the third mammary gland (I).

(2) CT lymphography of the mammary gland following interstitial injection of angiography contrast medium

The contrast medium entered the lymphatic channels almost immediately after injection and was distributed into the mammary lymphatic plexus. The efferent mammary lymphatic vessels and the lymph nodes into which they drained were usually visualized within 5 min after the injection. A schematic representation of the lymph drainage from each gland is presented in Fig. 1A, B and C. The images from the dogs (Dogs 1 and 2) that received Iopamiron-370 were clearer than those from the dog (Dog 3) that received Iopamiron-150, when contrast medium was injected into the subcutaneous portion of the mammary gland.

In Dog 1, contrast medium (Iopamiron-370) injected into the first and second mammary glands of both sides cranially drained into the ipsilateral axillary lymph nodes (Fig. 1A). However, some of the contrast medium from the right first mammary gland or the left second mammary gland simultaneously drained into the ipsilateral sternal lymphatic vessels and lymph nodes in the thoracic cavity (Fig. 1A and 2). Contrast medium from the third, fourth and fifth mammary glands on both sides usually drained into the ipsilateral superficial inguinal lymph nodes (Fig. 1A). Furthermore, the lymph path to the right superficial inguinal lymph node was extended forward into the left superficial inguinal lymph nodes via the pelvic lymphatic plexus in the pelvic cavity (Fig. 1A and 3), as often seen in anatomical specimens. In addition to this variation, the left third mammary gland also drained simultaneously cranially into the ipsilateral axillary lymph nodes and ipsilateral superficial inguinal lymph nodes and caudally into the medial iliac lymph nodes of the abdominal cavity (Fig. 1A and 4).

In Dog 2, contrast medium (Iopamiron-370) injected into the right second and third or left first and second mammary glands drained into the ipsilateral axillary lymph nodes (Fig. 1B). In particular, the right second mammary gland and the left first and second mammary glands also drained simultaneously into the ipsilateral sternal lymphatic vessels and nodes on the sternum in the thoracic cavity (Fig. 1B and 5). Furthermore, the right third mammary gland drained into the ipsilateral superficial inguinal lymph nodes (Fig. 1C).

In Dog 3, contrast medium (Iopamiron-150) injected into the right second mammary gland drained into the ipsilateral axillary lymph nodes, and the left fourth mammary gland drained into the ipsilateral superficial inguinal lymph nodes, and the flow continued into the medial iliac lymph nodes (Fig. 1C and 6).
Table 1. The relationship between India ink-injected site (mammary gland) and associated lymph nodes.

All the thoracic mammary glands of eight cases (A–H) constantly drain into the ipsilateral axillary lymph nodes (LNs). In the third or cranial abdominal mammary gland of three cases (I, J, K), two cases (I, J) drains into the ipsilateral axillary lymph nodes cranially and the superficial inguinal lymph nodes caudally simultaneously. In one case (I) of the two cases (I, J), the drainage reached into the contralateral inguinal lymph nodes via pelvic lymphatic plexus. While, K, injected into the third mammary gland only drained caudally into the ipsilateral superficial inguinal lymph nodes. The fourth or caudal abdominal mammary gland of five cases (L–P) and the fifth or inguinal mammary gland of two cases (Q, R) usually drain into the ipsilateral superficial inguinal lymph nodes and medial iliac lymph nodes in the center of the abdominal cavity. However, L, injected into fourth mammary gland, lymph drainage extend downward the contralateral inguinal lymph nodes via ipsilateral superficial inguinal lymph node and pelvic lymphatic plexus as seen in the third mammary gland (I).

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Dog1 (Fig. 1A): Iopamiron-370 injected into the first and second mammary glands of both sides drained cranially into the ipsilateral axillary lymph nodes. However, some of the contrast medium from the right first mammary gland or the left second mammary gland simultaneously drained into the ipsilateral sternal lymphatic vessels and lymph nodes. Contrast medium from the third, fourth and fifth mammary glands on both sides usually drained into the ipsilateral superficial inguinal lymph nodes. Furthermore, the path of the lymph to the right superficial inguinal lymph node was extended forward into the left inguinal lymph nodes. In addition to this variation, the left third mammary gland also simultaneously drained cranially into the ipsilateral axillary lymph nodes.

Fig. 1 (A,B,C): Pattern of drainage variations on canine mammary lymphography.
Variation and Visualization of the Lymphatic Drainage Routes in Normal Canine Mammary Glands. - Takuo Shida et al.

Fig. 2. 3D-CT image of the right second mammary gland and ipsilateral adjacent structures 5 minutes after intramammary injection of iopamiron-370 in Dog 1. Contrast medium from the mammary gland drained into the right axillary nodes (Yellow arrow). White arrow shows the lymphatic drainage route from the right second mammary gland.

Fig. 3. 3D-CT image of the right fourth mammary gland and adjacent structures 5 minutes after intramammary injection of iopamiron-370 in Dog 1. Contrast medium drained into the right superficial inguinal lymph node, and the path was extended forward into the left inguinal lymph nodes via the pelvic lymphatic plexus in the pelvic cavity, as often seen in anatomical specimens. White arrow shows the pelvic lymphatic vessel. Yellow arrows show right and left superficial inguinal lymph nodes.

Fig. 4. 3D-CT image of the left third mammary gland and ipsilateral adjacent structures 5 minutes after intramammary injection of iopamiron-370 in Dog 1. Contrast medium from the mammary gland drained simultaneously into the ipsilateral axillary lymph nodes cranially and ipsilateral superficial inguinal lymph nodes and medial iliac lymph nodes of the abdominal cavity caudally. White arrows show the lymphatic drainage route from the left third mammary gland. Yellow arrows show the axillary lymph nodes, superficial inguinal lymph nodes (back) and medial iliac lymph nodes (top).

Fig. 5. 3D-CT image of the right second mammary gland and ipsilateral adjacent structures 5 minutes after intramammary injection of iopamiron-370 in Dog 2. Contrast medium from the mammary gland drained into the right axillary nodes (Yellow arrow) on thorax and the right sternal lymph vessels (White arrow) in the thoracic cavity.
Discussion

The lymphatic drainage of healthy mammary glands has been extensively investigated from the anatomical point of view [1, 3, 8-12] and by radiographic techniques [4, 6, 7], and the efferent mammary lymphatics, as well as the lymph nodes into which they drain, have been described in detail. On the other hand, mammary tumors are known to sometimes alter the lymphatic drainage pattern, forming new draining channels and recruiting a large number of lymph nodes [2, 5, 8, 13].

The present study investigated the lymphatic drainage patterns of healthy canine mammary glands using an anatomical approach and examined the usefulness of CT lymphography of canine mammary glands in clinical practice. Although the lymphatic drainage pattern from each mammary gland of the dogs was consistent with that described previously [1, 10, 11], there were variations between individuals in the present study. For instance, the second mammary gland usually drained into the ipsilateral axillary lymph nodes, but sometimes it also drained into the sternal lymph vessels and lymph nodes in the thoracic cavity. The participation of the sternal lymph nodes as sentinel lymph nodes of the first and second neoplastic mammary glands has been reported previously by Pereira et al. [8] and Patsikas et al. [5]. They described that this lymphatic variation was only present in the drainage of neoplastic glands but not in that of healthy mammary glands. However, we found the same route of drainage in healthy mammary glands, as mentioned above. In addition, Pereira et al. [8] described that the contralateral axillary lymph nodes were sentinel lymph nodes of healthy thoracic mammary glands. However, we were unable to identify this route in the present study. In contrast, the fourth mammary gland usually drained into the ipsilateral superficial inguinal lymph nodes; however, sometimes it also drained into the contralateral superficial inguinal lymph nodes. This lymphatic drainage pattern has not been described previously.

The lymphatic system is considered a major route for metastases of canine mammary cancer, and surgery for mammary tumors often implies removal of the tumor along with the gland-associated lymph nodes. To achieve successful, appropriate surgical excision of mammary tumors, an accurate assessment of the glands associated with lymphatic drainage along...
Variation and Visualization of the Lymphatic Drainage Routes in Normal Canine Mammary Glands.-Takuo Shida et al.

with involved lymph nodes is required in order to determine the removal site, and whether the gland-associated lymph nodes can be clearly delineated is key to this process.

In this study, CT lymphography with the two warmed water-soluble angiographic contrast media (iopamidol: Iopamiron-370, high consistency medium; and Iopamiron-150, low consistency medium) was used successfully for distinct visualization of the efferent mammary lymphatics and associated lymph nodes in dogs. Specifically, the best time to obtain clear images for CT lymphography was found to be 5 min after injection and massage, with no individual differentiation among the three dogs in this study. Furthermore, the images from the dogs that received Iopamiron-370 were clearer than those from the dog that received Iopamiron-150, when contrast medium was injected into the subcutaneous portion of the mammary gland. However, it was also possible to use Iopamiron-150 for adequate distinct visualization of lymphatics and nodes.

We observed clear CT contrast images of the lymphatic tract from each mammary gland by injection of small doses of contrast medium into the subcutaneous portions near/under the teat of each mammary gland. CT lymphography allowed accurate localization of the lymphatic pathways. Moreover, the topographic 3D-CT images provided detailed anatomical information on individual lymphatic channels and nodes, without overlaps with the surrounding structures, such as bones (ribs and thoracic vertebrae) and adjacent soft tissues, while clearly depicting the lymph course. Thus, CT images are useful for visualization of the 3D positions and courses of the efferent lymphatic vessels and lymph nodes, thereby enabling both appropriate surgical excision and accurate assessment of postsurgical prognosis.

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References

正常な犬の乳腺のリンパ流路パターンと可視化に関する解剖学的研究

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和文要約
健康犬の乳腺からのリンパ流路について調べた。解剖学的研究では、リンパ流路の多くが定型的パターンを示したが、個体により変異も見られた。CT分析では、造影剤を乳腺皮下に少量注射すると非侵襲性で迅速にリンバ経路を可視化することができた。これらのことから、臨床では各個体で、3D-CTによるリンバ造影をすることが、変異のあるリンバ流路の特定が容易になり、その結果、その個体にあたる最も適切な乳腺腫瘍の外科切除と予後判定の実施が可能になると思われた。

Key word: Anatomical study, Dog, Lymph Drainage Route, Mammary Glands, 3D-CT