Lower-Limb Lymphatic Drainage Pathways and Lymph Nodes: A CT Lymphangiography Cadaver Study

Akira Shinaoka, MD, PhD • Seijiro Koshimune, MD • Hiroo Suami, MD, PhD • Kiyoshi Yamada, MD, PhD • Kanae Kumagishi, PhD • John Boyages, MD, PhD • Yoshihiro Kimata, MD, PhD • Aiji Ohtsuka, MD, PhD

From the Department of Human Morphology (A.S., K.K., A.O.) and Department of Plastic and Reconstructive Surgery (A.S., S.K., K.Y., Y.K.), Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Science, 2-5-1 Shikata-cho, Kita-ku, Okayama-shi 700-8558, Japan; and Faculty of Medicine and Health Sciences, Macquarie University, Sydney, Australia (H.S., J.B.). Received May 29, 2019; revision requested July 17; revision received September 14; accepted October 2. **Address correspondence to** A.S. (e-mail: *a-shinaoka@okayama-u.ac.jp*).

This work was supported by the Japan Society for the Promotion of Science via Grants-in-Aid for Scientific Research (KAKENHI Award Number: 16K20358) and Wesco Research Foundation.

Conflicts of interest are listed at the end of this article.

See also the editorial by Weiss in this issue.

Radiology 2019; 00:1–8 • https://doi.org/10.1148/radiol.2019191169 • Content code: VA

Background: Most lymphatic imaging examinations of the lower limb require intradermal or subcutaneous injection of tracer material into the foot to demonstrate the lymphatic vessels; however, no standard protocol exists, and single or multiple injections are applied at different sites.

Purpose: To determine the three-dimensional relationships between each lymphatic group of the lower limb and corresponding regional lymph nodes.

Materials and Methods: A total of 130 lower limbs (55 from men and 75 from women) from 83 fresh human cadavers were studied. Lymphatic vessels were first visualized by using indocyanine green fluorescent lymphography with 19 injection sites in the foot, classified into four distinct lymphatic groups (anteromedial, anterolateral, posteromedial, and posterolateral); dilute oil-based contrast material was then injected. Next, specimens were scanned with CT and three-dimensional images were analyzed.

Results: The anteromedial and anterolateral lymphatic groups of the lower-leg lymphatic vessels were independent of each other and connected to different regional lymph nodes in the inguinal region. The posteromedial group and the anteromedial group in the lower leg drained to the same inguinal lymph nodes. Only the posterolateral group of lymphatic vessels in the lower leg drained to the popliteal lymph nodes. Leg lymphatic drainage pathways were independent of genital pathways.

Conclusion: Standard injection sites at the web spaces between the toes did not help visualize some lymph nodes of the lower leg. Additional injection sites in the medial, lateral, and posterior aspect of the foot would be better for evaluating the whole lymphatic pathways and regional lymph nodes and for improving understanding of leg lymphedema.

Online supplemental material is available for this article.

Imaging of the lymphatic vessels is an essential tool for diagnostic assessment of lymphedema. Lymphoscintigraphy is the current reference standard for lymphatic imaging (1–3). SPECT (4), indocyanine green (ICG) fluorescent lymphography (5–7), and MR lymphography (8) are emerging techniques. Lymphatic imaging of the lower limb requires intradermal or subcutaneous injection of tracer or contrast material in the foot to demonstrate the lymphatic vessels. No standard protocol exists, and single or multiple injections are applied at different sites.

Each lymphatic vessel has an independent origin in the foot. The lymphatic vessel often branches and converges throughout its course, but it is uncommon to have interconnections with adjacent lymphatic vessels to produce a network. These characteristics allow us to categorize the lymphatic pathways in the lower limb into groups. Conventionally, the lymphatic pathways are divided into two groups: an anteromedial group connecting to the superficial inguinal lymph nodes and a posterolateral group connecting to the popliteal lymph nodes (9,10).

Few anatomic studies have addressed the lymphatic vessels because of technical difficulties with their identification. Kinmonth and Eustace (11) developed lymphangiography and found that leg lymphedema is caused by deterioration of lymphatic vessels, lymph nodes, or both and that the pathologic process arose first in the lymph node. However, current lymphatic imaging protocols may miss information because of the lack of anatomic knowledge regarding the relationship between lymphatic vessels and their regional lymph nodes (12). Investigators of a previous study of ICG fluorescent lymphography in cadavers (13) classified lymphatic pathways into four distinct groups in the lower limb: anteromedial, anterolateral, posteromedial, and posterolateral. Origins of the lymphatic vessels in the foot may be defined in relationship to these four lymphatic groups, which could be selectively visualized with ICG lymphography.

A limitation of previous studies was that the relationship between a lymphatic group and its regional lymph nodes could not be determined with ICG lymphography

Abbreviations

GSV = great saphenous vein, ICG = indocyanine green

Summary

This study identified suitable tracer injection sites for comprehensive imaging of the lower limb to help evaluate leg lymphedema.

Key Results

- There are four lymphatic pathways in the lower leg (anteromedial, anterolateral, posterolateral, and posteromedial) that drain mainly to three lymph nodes; two nodes were in the superficial inguinal region and one node was in the popliteal region.
- Three lymph nodes in two inguinal regions (inferior lateral 1 and inferior lateral 2) and one lymph node in the popliteal region (superficial popliteal) receive almost three-quarters (73%) of the lymphatic drainage of the lower leg.
- The posterolateral lymphatic vessels of the lower leg were the only group connecting to the popliteal lymph node.
- Conventional lymphangiography injection protocols that use only injections into toe web spaces demonstrated only the anteromedial pathway and did not depict other lymphatic pathways.

because of its limited penetration depth of about 2 cm from the skin surface; ICG lymphography could depict the lymphatic vessels below the knee but could not consistently depict those in the thigh. To address this limitation, in this study we used CT lymphangiography to elucidate a detailed three-dimensional anatomy of lymphatic vessels in the lower limbs. We also sought to determine whether there is a consistent relationship between each lymphatic group in the lower limb and the regional lymph nodes.

Materials and Methods

One hundred thirty lower limbs (55 from men and 75 from women) from 83 fresh human cadavers were studied. Cadavers with lymphedema, scars, or an amputated limb were excluded. The cadavers were transported to the anatomy laboratory in the Department of Human Morphology, Okayama University, at the earliest opportunity. All donors had provided written wills to donate their bodies for medical education and research. This study was approved by the ethics committee at Okayama University Graduate School of Medicine (reference K1605–020). ICG lymphography was used to identify the lymphatic vessels, and CT was used to help determine the three-dimensional relationship between the lymphatic vessels and their regional lymph nodes.

ICG Lymphography

ICG, 25 mg (Diagnogreen; Daiichi Sankyo, Tokyo, Japan), was diluted with 10 mL of pure water. Injection sites were marked around the foot along the border between the dorsum and planta. A total of 19 injection sites in the foot were selected (Fig 1), and 0.05 mL of ICG solution was subcutaneously injected by using a 1-mL syringe with a 30-gauge needle. Immediately after the injections, gentle hand massage was applied at each injection site and then to the lower limb. The lymphatic vessels were identified with a near-infrared camera system (ASI224MC; Suzhou ZWO, Suzhou, China) (excitation: 760 nm; emission: 830 nm), and massage was continued until ICG reached above the knee (14). Sequential still images were obtained with a camera slider system, and montages of the images were made with imaging software (Image Composite Editor; Microsoft, Redmond, Wash). The lymphatic vessels in the lower limb were classified into four lymphatic groups: anteromedial, anterolateral, posteromedial, and posterolateral (Fig 2) (assessed by A.S. and S.K, with 10 and 12 years of experience in lymphography, respectively) as detailed in a previous report (15).

CT Lymphangiography

Iodized oil (Lipiodol; Guerbet Japan, Tokyo, Japan) solution (20%) diluted with diethyl ether (Tokyo Chemical Industry, Tokyo, Japan) was used as the contrast material. The skin around the ankle was incised carefully under a microscope, and identified lymphatic vessels were cannulated with a 32-gauge needle. Iodized oil solution was injected gently with a 2-mL glass syringe. The amount of injection was usually more than 1 mL per lymphatic vessel, and injection was stopped after leakage from the needle hole was noted.

CT was performed with a multisection scanner (Alexion TSX-032A; Toshiba Medical Systems, Tochigi, Japan) by using axial scanning (120 kVp; 100 mA; slice thickness, 1.0 mm; slice interval, 0.5 mm; field of view, 319; speed, 0.75). CT images were transferred to an image workstation (Virtual Place; AZE, Kawasaki, Japan) to characterize iodized oil–enhanced lymphatic vessels and lymph nodes (Figs 3, 4).

In the first study of 10 lower-limb specimens (eight right legs and two left legs), all lymphatic vessels were cannulated and injected with the contrast material. In the second study, only one lymphatic group was injected with contrast material per specimen. Thirty legs were used to investigate each lymphatic group, and a total of 120 cadaver specimens were used for all four groups.

The superficial inguinal lymph nodes were categorized into five regional groups (four quadrants and central) divided by the great saphenous vein (GSV) and a horizontal line at the saphenofemoral junction. A previous anatomic study named these regional groups as superior medial, superior lateral, inferior medial, inferior lateral, and central (Fig 5) (12). We further grouped the lymph nodes in the inferior lateral or inferior medial groups into three lymph node groups. We coded lymph nodes along the GSV as inferior lateral 1 or inferior medial 1, those near the horizontal line as inferior lateral 3 or inferior medial 3, and those in between as inferior lateral 2 or inferior medial 2. The deep inguinal lymph nodes were coded as deep inguinal, and they were located in the deep aspect close to the femoral artery and vein. Superior lateral, superior medial, and central lymph node groups were not categorized into subgroups. The popliteal lymph nodes were divided into two groups separated by the deep fascia: superficial popliteal (Fig 5) and deep popliteal. In total, there were 10 potential inguinal locations and two popliteal locations.

We documented the locations of the first-tier nodes that each lymphatic vessel group initially reached and the location of subsequent second-tier nodes. We also measured the short axis of each identified lymph node on the CT image and the distance

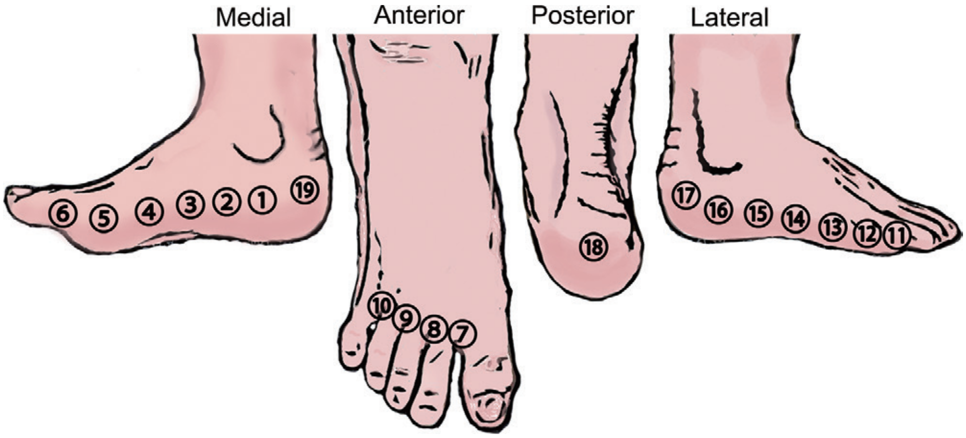

Figure 1: Schematic shows injection points of indocyanine green around the circumference of the foot (Reprinted, with permission, from reference 13).

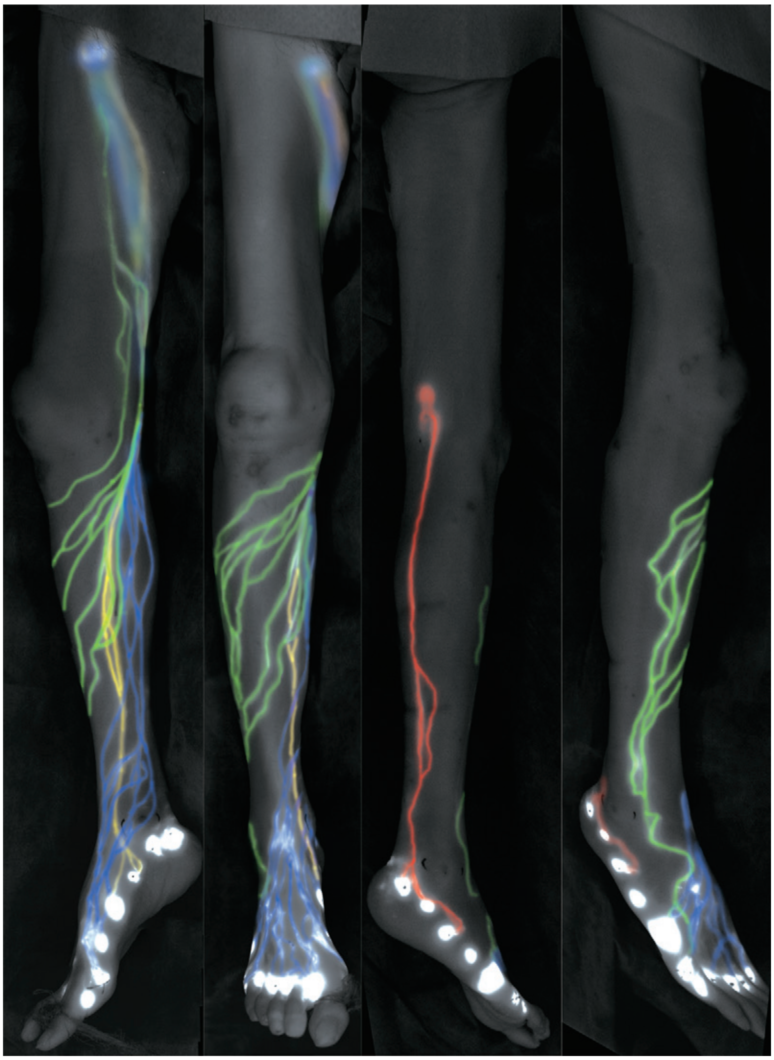

Figure 2: Images from indocyanine green fluorescent lymphography in single lower limb in cadaver specimen. Lymphatic vessels were divided into four groups according to the quadrant in which the vessels traversed at the ankle: posteromedial (yellow), anteromedial (blue), anterolateral (green), and posterolateral (red).

between the center of each node and the horizontal plane at the saphenous hiatus (A.S. and S.K., with 10 and 15 years of experience in CT, respectively).

Results

Lymphatic Vessel Study

In the first part of our study, involving 10 cadaver specimens, we mapped and recorded the lymphatic vessels with ICG fluorescent lymphography on the CT images for all entire courses from the injection sites to the inguinal and popliteal lymph nodes (Fig 3).

Lymphatic vessels within the same group divided and merged frequently and had occasional connections with the other group. The posterolateral group did not show any connections with the other three groups. The lymphatic vessels in the other three lymphatic groups were distinguishable and independent in the lower leg, but they gradually gathered above the knee and ran in a bundle along the GSV in the thigh. Thus, we were unable to elucidate the relationship between each lymphatic group and its regional lymph nodes in the inguinal region.

With selective injection into the lymphatic vessels of each individual group (study 2: 30 cadaveric specimens per lymphatic group, 120 specimens in total), we successfully identified which lymphatic vessel connected to which lymph nodes.

Posteromedial Group

One or two lymphatic vessels in this group were identified immediately above the deep fascia close to the GSV at the posteromedial aspect of the ankle level (Figs 4, *A,* E1 [online]). They ran along the main trunk of the GSV, and usually one or two vessels connected to lymph nodes in the inguinal region (Fig E2 [online]). The lymphatic vessels crossed over the GSV in the middle thigh to the lateral side of the GSV. They sometimes branched both in the lower leg and the thigh around the knee. These branches sometimes entered into the Hunter (adductor) canal located at the medial aspect of the middle third of the thigh and ran below the deep fascia to become the deep lymphatic vessels (six of 30, 20.0%).

Anteromedial Groups

One to five lymphatic vessels in this group were identified at the anteromedial aspect of the ankle level (Figs 4, *B,* E3 [online]). The lymphatic vessels in this group ran in the anteromedial aspect of the lower leg. Their course was a gentle sigmoidal curve parallel to the cutaneous branches of the GSV in the lower leg and to the main trunk of the GSV in the thigh (Fig E2 [online]). They ran

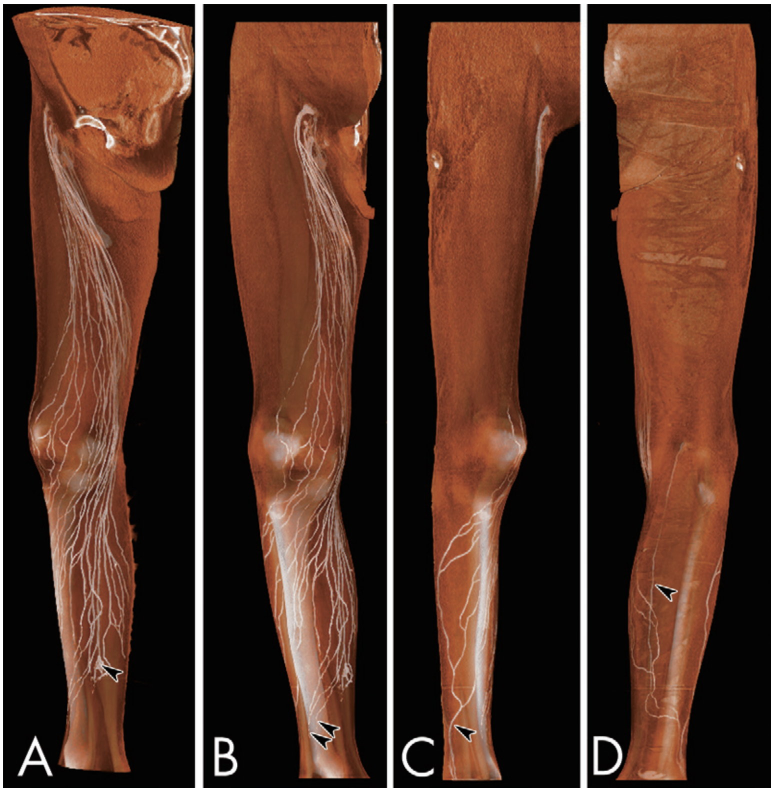

Figure 3: CT lymphangiographic images of all lymphatic vessels in one cadaveric lower limb in the first study. Arrowheads indicate, *A*, posteromedial, *B*, anteromedial, *C*, anterolateral, and, *D*, posterolateral groups.

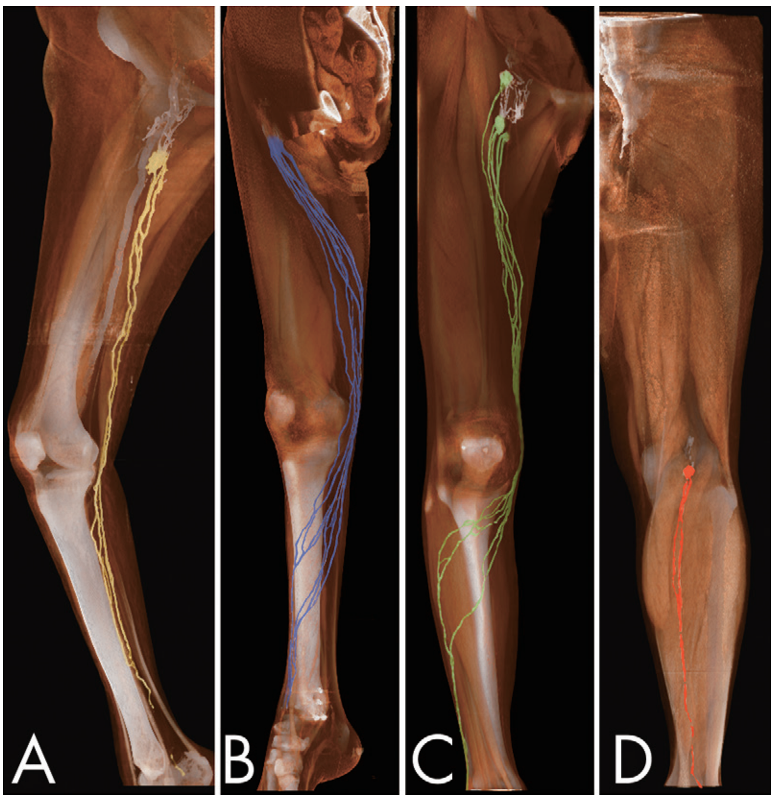

Figure 4: CT lymphangiographic images obtained with selective lymphatic group injection in four differerent legs of cadaver specimens. One selected lymphatic group was injected with contrast material in each specimen, as follows: *A*, posteromedial (yellow), *B*, anteromedial (blue), *C*, anterolateral (green), and, *D*, posterolateral (red).

along the lateral side of the GSV in the upper thigh and converged immediately before connecting to their regional nodes. They never branched to the deep lymphatic vessels but remained superficially above the deep fascia.

Anterolateral Group

One or two lymphatic vessels in this group were found in the middle of the subcutaneous fat layer at the anterolateral aspect of the ankle (Figs 4, *C*, E4 [online]). They traveled in the anterolateral aspect of the lower leg and gradually changed their course toward the anteromedial aspect. The lymphatic vessels in the anterolateral group were independent from the anteromedial group in the lower leg. They ran along the lateral side of the GSV and on the sartorius muscle in the proximal thigh. They never branched to the deep lymphatic vessels and stayed superficially above the deep fascia.

Posterolateral Group

One or two lymphatic vessels in this group were found in the posterolateral aspect at the ankle level (Figs 4, *D*, E5 [online]). They traveled along the lesser saphenous vein and connected to the popliteal lymph nodes at the popliteal fossa. They sometimes branched and connected to the anteromedial or anterolateral group (five of 30, 16.7%). Efferent lymphatic vessels from the popliteal lymph nodes ran along the deep femoral artery to connect with the deep lymphatic vessels in most specimens (29 of 30, 96.7%). Efferent lymphatic vessels in one specimen never went under the deep fascia and stayed superficially above the deep fascia in the thigh (one of 30, 3.3%).

Lymph Node Study

A total of 232 first-tier lymph nodes were identified in the second part of the study, which used 120 lower extremities. The locations of the nodes, according to frequency, were as follows: inferior lateral (73 of 232, 67.2%), inferior medial (30 of 232, 12.9%), popliteal (30 of 232, 12.9%), superior lateral (12 of 232, 5.2%), and deep inguinal (four of 232, 1.7%) (Table). There were no nodes in the superior medial region. The number of first-tier nodes in inferior lateral 1, inferior lateral 2, inferior lateral 3, inferior medial 1, inferior medial 2, inferior medial 3, and superficial popliteal locations were consistent, and only one lymph node was identified per specimen. However, the number of first-tier nodes in superior lateral and deep popliteal locations varied from one to three.

Measurements of the short axis of the first-tier nodes are summarized in the Table. Inferior lateral 1 was the largest lymph node among all groups. Inferior lateral 1, inferior lateral 2, inferior medial 1, and inferior medial 2 lymph nodes were located at a median of 36.8 mm (range, 8.9–74.9 mm), 28.6 mm (range, 5.4–57.3 mm), 39.2 mm (range, 13.5–49.8 mm), and 13.1 mm (range, 4.0–28.0 mm) below the saphenous hiatus, respectively. The superior lateral lymph node was located a median of 16.5 mm (range, 9.0–30.0 mm) above the saphenous hiatus.

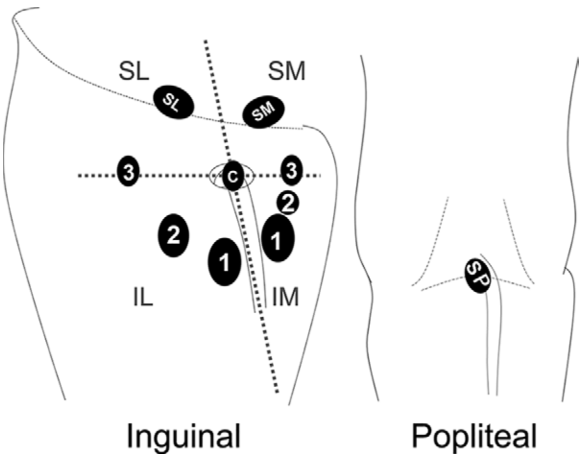

Figure 5: Schematic illustrates right inguinal lymph nodes (left) and popliteal lymph node (right). Inguinal lymph nodes are divided into five regions (four quadrants and a central region [C]) by a vertical dotted line on the great saphenous vein and a horizontal dotted line at femoral hiatus. Superior medial (SM), superior lateral (SL), inferior medial (IM), inferior lateral (IL), and central regions are noted. Inferior lateral and inferior medial regions were further divided into subregions adjacent to the saphenous vein (inferior lateral 1, inferior medial 1), near the horizontal axis (inferior lateral 3, inferior medial 3), and in between (inferior lateral 2 or inferior medial 2). The superficial popliteal lymph nodes (SP) were located above the deep fascia.

First-Tier Nodes in 120 Cadavers

Node Location	No. of Nodes (n = 232)*	No. of Nodes per Location	Median Size (mm)†
Superior lateral	12 (5.2)	1–3	11.7 (5–15.5)
Superior medial	0 (0)	0	NA
Inferior lateral	156 (67.2)		
Region 1	92 (39.7)	1	11.6 (5.4–21.1)
Region 2	51 (22.0)	1	9.3 (6.2–13.8)
Region 3	13 (5.6)	1	7 (5.2–9)
Inferior medial	30 (12.9)		
Region 1	22 (9.5)	1	8.9 (6.2–14.2)
Region 2	7 (3.0)	1	5.8 (4.7–11.8)
Region 3	1 (0.4)	1	5.6 (NA)
Central	0 (0)	0	NA
Deep inguinal	4 (1.7)	1	5.4 (4.3–7.2)
Popliteal	30 (12.9)		
Superficial	26 (11.2)	1	8.0 (5.3–13.4)
Deep	4 (1.7)	1–3	10.7 (5.7–14.3)

* Numbers in parentheses are percentages.

† Measurements were obtained in the short axis. Numbers in parentheses are the range. NA = not applicable.

Correlation between Each Lymphatic Vessel Group and Lymph Node Region

Correlations between each lymphatic vessel group and lymph node region for first-tier nodes and for first-tier plus second-tier nodes are summarized in Figure 6 and Figure E6 (online), respectively. Figure 7 shows the relationship between lymphatic groups and lymph nodes. The posterolateral group only reached to the superficial popliteal lymph node, and the efferent lymphatic vessel from the superficial popliteal lymph node connected to the deep popliteal lymph node. Some branches of the posterolateral group reached the inferior lateral 1 and inferior lateral 2 lymph nodes. Lymphatic vessels in posteromedial and anteromedial groups reached to the same regional lymph nodes: inferior lateral 1, inferior lateral 2, and inferior medial 1. Branches of the posteromedial group sometimes reached to the deep inguinal lymph node; the anteromedial group never reached the deep inguinal lymph nodes directly. The anterolateral group reached the inferior lateral lymph nodes and superior lateral lymph nodes, but they never connected to the inferior medial lymph nodes.

Discussion

This study demonstrates the three-dimensional detailed anatomy of the course of the subcutaneous lymphatic vessels from the ankle to the regional inguinal or popliteal lymph nodes in cadaver lower-limb specimens. The data obtained from multiple fresh cadaver specimens confirmed that the lymphatic vessels in the lower limb are divided into four distinct lymphatic groups. Our findings of the relationship between each lymphatic vessel group and their corresponding regional lymph nodes suggested that three lymph nodes in two inguinal regions (inferior lateral 1 and inferior lateral 2) and one in the popliteal region (superficial popliteal) have a vital role in receiving almost three-fourths (169 of 232, 72.8%) of the lymphatic drainage of the lower limb. These results will help guide more standardized injection sites based on human anatomy to image the inguinal and popliteal lymph nodes. They also provide a detailed normal lymphatic image to help lymphatic interpretation in pathologic conditions, particularly lymphedema. They may also help guide sentinel node mapping, surgical planning of skin removal, and radiation therapy planning to reduce the incidence of lymphedema.

Metastasis to the popliteal lymph node is possible in patients with malignant melanoma in distal lower limbs. The reported prevalence of identification of the popliteal lymph node during sentinel node biopsy ranges from 1% to 20% (16). Conventional anatomic mapping showed that drainage from the posterolateral aspect of the heel, sole, and lateral malleoli follows the lesser saphenous vein to the popliteal lymph node. The posterolateral group was the only group connecting to the popliteal lymph node from the posterolateral lymphatic territory in the foot (Figs 4–6, E5, E6 [online]). It may be useful to carefully image the popliteal lymph node with malignancies in this tributary by using US, CT, MRI, or SPECT lymphoscintigraphy before sentinel lymph node biopsy.

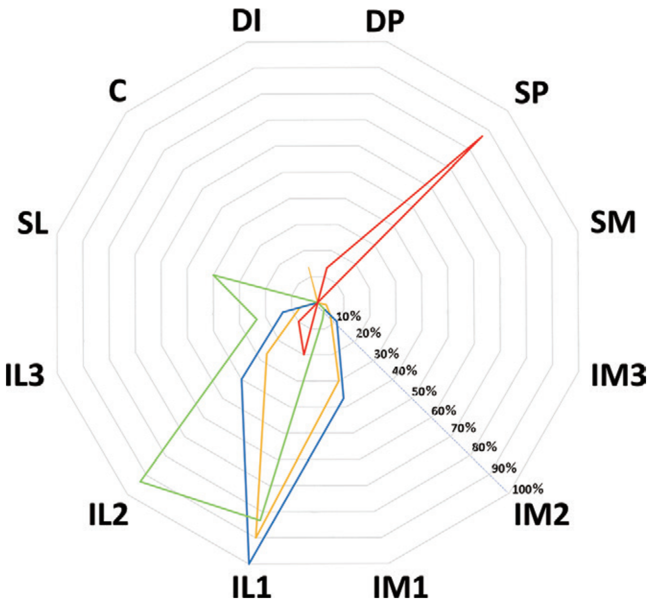

Figure 6: Radar chart shows correlation between lymphatic group and location of the first-tier node. Percentages represent the detection rate of the first-tier node in each lymphatic group. Lymphatic vessel groups are color-coded as follows: posteromedial (yellow), anteromedial (blue), anterolateral (green), and posterolateral (red). Three regional lymph nodes received most of the lymphatic fluid in lower limbs: inferior lateral (IL) 1 and 2 and superficial popliteal (SP). C = central, DI = deep inguinal, DP = deep popliteal, IM = inferior medial, SL = superior lateral, SM = superior medial.

Investigators of a previous study using a cadaver model proposed that the lymphatic vessels in the leg were divided into four independent groups instead of the conventional two groups that connected to the inguinal or popliteal lymph nodes (15). Because of limited information about past anatomic studies, it has been widely misunderstood that the lymphatic vessels are a full connected network system, similar to veins. This may be why current lymphoscintigraphy protocols use only a single tracer injection site, commonly at the web space between the first and second toes (2,17). According to our results, injection into this single web space demonstrates the lymphatic vessels only in the anteromedial group. The anteromedial and posteromedial groups did overlap in the leg, but the anterolateral and posterolateral groups were independent from them.

Although the posteromedial group had the same lymph node distribution as the anteromedial group, it ran in a different layer from the anteromedial group. Recent embryologic findings elucidated that development of the lymphatic system was regulated by peripheral blood vessels (18,19). Our findings in this study demonstrated that lymphatic vessels in the anteromedial group were accompanied by the GSV branches and those in the posteromedial group were accompanied by the GSV trunk. Therefore, we speculate that development of anteromedial and posteromedial groups is regulated by the branching pattern of the GSV and that these two lymphatic groups may have a complementary relationship. On the contrary, the posteromedial group had connection only with the deep subfascial lymphatic vessels. This superficial and deep connection may function as a detour route to prevent lymphedema when the superficial lymphatic pathway was blocked.

We found that most lymphatic vessels in the leg were connected to only three lymph nodes (labeled as inferior lateral 1, inferior lateral 2, and superficial popliteal in Fig 5). SPECT has been used to identify locations of sentinel lymph nodes for vulvar or penile cancer (20,21). These results suggested that lymphatic drainage from the genital area reached to the superior medial lymph node most commonly and also to other lymph nodes (superior lateral, central, and inferior medial lymph nodes) but rarely reached the inferior lateral lymph node. Our study helps improve understanding of the functional division of inguinal lymph nodes in the five regions. In addition, lymph node size might indicate lymphatic function in the lower limb; the inferior

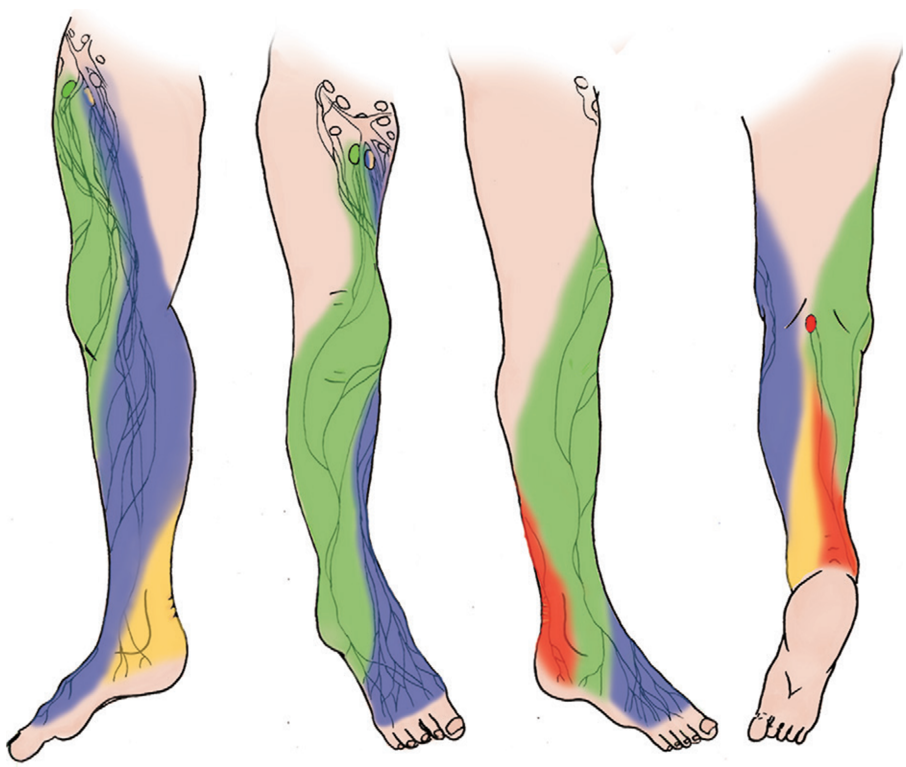

Figure 7: Schematic shows lymphatic groups in the lower limb: posteromedial (yellow), anteromedial (blue), anterolateral (green), and posterolateral (red) groups.

lateral 1 lymph node was dominant (largest node), and inferior lateral 2 and superficial popliteal lymph nodes were supplemental. The sentinel nodes in the lower limb were separate from those in the genital region; therefore, sentinel node biopsy for genital cancer should not affect leg lymphatic drainage and cause lymphedema unless there are cancer metastases in more proximal lymph nodes.

In previous lymphoscintigraphic studies, the diagnostic criteria for impairment of lymphatic function included delayed visualization, nonvisualization, or reduced number of lymph nodes. Duration of tracer concentration at regional lymph nodes was used for quantitative analysis (22–28). However, the evaluation of tracer uptake in the original lymph node in these studies was difficult in leg lymphedema because of alteration of the lymphatic vessels. Specification of the original lymph nodes in our study may help refine quantitative lymphoscintigraphy for the lower limb to focus on the function of these lymph nodes.

The most common site of tracer injection for lymphatic imaging has been the web space between the first and second toes, with the web space between the second and third toes as an alternative (29). Injections into multiple toe web spaces have also been reported (1,3,22). Our study and a previous study (12) confirmed that toe web space injections demonstrated only the anteromedial pathway and missed other pathways. We propose that two additional injection sites (sites 14 and 16 in Fig 1) are required for analyzing the anterolateral group and their regional lymph nodes (inferior lateral 2) and the posterolateral group and their node (superficial popliteal). The combination of injection sites 7 (or 1), 14, and 16 would be appropriate for analyzing the three specific lymph nodes for the lower limb.

A limitation of our study was that materials were cadaver specimens. In future studies, we plan to use the new protocol with these injection sites in patients with lymphedema to confirm translational capability of the cadaver study to a clinical setting.

In conclusion, we investigated the anatomy of lymphatic vessels in the lower limb by using 130 cadaveric lower limbs. We found that four distinct lymphatic groups took the roles of lymphatic drainage in the lower limb and connected to three first-tier nodes. These results should prompt reconsideration of tracer injection sites and evaluation of lymph nodes for patients with lower-limb or genital malignancy and improve lymphatic imaging for patients with lower-limb lymphedema.

Author contributions: Guarantors of integrity of entire study, A.S., J.B., Y.K., A.O.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; approval of final version of submitted manuscript, all authors; agrees to ensure any questions related to the work are appropriately resolved, all authors; literature research, H.S., J.B., Y.K., A.O.; clinical studies, K.Y., J.B.; experimental studies, A.S., S.K., K.K., Y.K., A.O.; statistical analysis, A.S., K.K.; and manuscript editing, A.S., H.S., K.Y., K.K., J.B., Y.K., A.O.

Disclosures of Conflicts of Interest: **A.S.** disclosed no relevant relationships. **S.K.** disclosed no relevant relationships. **H.S.** disclosed no relevant relationships. **K.Y.** disclosed no relevant relationships. **K.K.** disclosed no relevant relationships. **J.B.** disclosed no relevant relationships. **Y.K.** disclosed no relevant relationships. **A.O.** disclosed no relevant relationships.

References

1. Witte CL, Witte MH, Unger EC, et al. Advances in imaging of lymph flow disorders. RadioGraphics 2000;20(6):1697–1719.
2. Notohamiprodjo M, Weiss M, Baumeister RG, et al. MR lymphangiography at 3.0 T: correlation with lymphoscintigraphy. Radiology 2012;264(1):78–87.
3. Maegawa J, Mikami T, Yamamoto Y, Satake T, Kobayashi S. Types of lymphoscintigraphy and indications for lymphaticovenous anastomosis. Microsurgery 2010;30(6):437–442.
4. Baulieu F, Bourgeois P, Maruani A, et al. Contributions of SPECT/CT imaging to the lymphoscintigraphic investigations of the lower limb lymphedema. Lymphology 2013;46(3):106–119.
5. Unno N, Inuzuka K, Suzuki M, et al. Preliminary experience with a novel fluorescence lymphography using indocyanine green in patients with secondary lymphedema. J Vasc Surg 2007;45(5):1016–1021.
6. Shinaoka A, Koshimune S, Yamada K, et al. Accelerated lymph flow in early-stage secondary lymphedema detected by indocyanine green fluorescence lymphography. J Reconstr Microsurg 2017;33(8):596–602.
7. Matsumoto K, Shinaoka A, Yamada K, Kimata Y. Exercise-loaded indocyanine green fluorescence lymphangiography for diagnosing lymphedema. J Reconstr Microsurg 2019;35(2):138–144.
8. Lohrmann C, Foeldi E, Speck O, Langer M. High-resolution MR lymphangiography in patients with primary and secondary lymphedema. AJR Am J Roentgenol 2006;187(2):556–561.
9. Tubs RS. Pelvic girdle and lower limb. In: Starling S, ed. Gray's Anatomy. 41st ed. Amsterdam, the Netherlands: Elsevier, 2016; 1320–1321.
10. Yamazaki S, Suami H, Imanishi N, et al. Three-dimensional demonstration of the lymphatic system in the lower extremities with multi-detector-row computed tomography: a study in a cadaver model. Clin Anat 2013;26(2):258–266.
11. Kinmonth JB, Eustace PW. Lymph nodes and vessels in primary lymphoedema: their relative importance in aetiology. Ann R Coll Surg Engl 1976;58(4):278–284.
12. Poirier P, Cuneo B. Special study of the lymphatic of the body. In: Delamere G, ed. The Lymphatics. General Anatomy of the Lymphatics. Westminster, England: Archibald Constable & Co Ltd, 1903; 115–117.
13. Shinaoka A, Koshimune S, Yamada K, et al. Correlations between tracer injection sites and lymphatic pathways in the leg: a near-infrared fluorescence lymphography study. Plast Reconstr Surg 2019;144(3):634–642.
14. Kitai T, Inomoto T, Miwa M, Shikayama T. Fluorescence navigation with indocyanine green for detecting sentinel lymph nodes in breast cancer. Breast Cancer 2005;12(3):211–215.
15. Shinaoka A, Koshimune S, Yamada K, et al. A fresh cadaver study on indocyanine green fluorescence lymphography: a new whole-body imaging technique for investigating the superficial lymphatics. Plast Reconstr Surg 2018;141(5):1161–1164.
16. Nijhuis AAG, de A O Santos Filho ID, Uren RF, Thompson JF, Nieweg OE. Clinical importance and surgical management of sentinel lymph nodes in the popliteal fossa of melanoma patients. Eur J Surg Oncol 2019;45(9):1706–1711.
17. Bourgeois P. Scintigraphic investigations of the lymphatic system: the influence of injected volume and quantity of labeled colloidal tracer. J Nucl Med 2007;48(5):693–695.
18. Hogan BM, Black BL. Developmental biology: diversity in the lymphatic vasculature. Nature 2015;522(7554):37–38.
19. Liu X, Uemura A, Fukushima Y, Yoshida Y, Hirashima M. Semaphorin 3G provides a repulsive guidance cue to lymphatic endothelial cells via neuropilin-2/plexinD1. Cell Reports 2016;17(9):2299–2311.
20. Collarino A, Donswijk ML, van Driel WJ, Stokkel MP, Valdés Olmos RA. The use of SPECT/CT for anatomical mapping of lymphatic drainage in vulvar cancer: possible implications for the extent of inguinal lymph node dissection. Eur J Nucl Med Mol Imaging 2015;42(13):2064–2071.
21. Leijte JA, Valdés Olmos RA, Nieweg OE, Horenblas S. Anatomical mapping of lymphatic drainage in penile carcinoma with SPECT-CT: implications for the extent of inguinal lymph node dissection. Eur Urol 2008;54(4):885–890.
22. Szuba A, Shin WS, Strauss HW, Rockson S. The third circulation: radionuclide lymphoscintigraphy in the evaluation of lymphedema. J Nucl Med 2003;44(1):43–57.
23. Nganga EC, Gitau S, Makhdomi K. Lower limb lymphoscintigraphy patterns among patients with lower limb lymphedema: a pictorial essay. Clin Transl Imaging 2018;6(2):135–143.
24. Carena M, Campini R, Zelaschi G, Rossi G, Aprile C, Paroni G. Quantitative lymphoscintigraphy. Eur J Nucl Med 1988;14(2):88–92.
25. Bräutigam P, Földi E, Schaiper I, Krause T, Vanscheidt W, Moser E. Analysis of lymphatic drainage in various forms of leg edema using two compartment lymphoscintigraphy. Lymphology 1998;31(2):43–55.
26. Kim P, Lee JK, Lim OK, Park HK, Park KD. Quantitative lymphoscintigraphy to predict the possibility of lymphedema Development after breast cancer surgery: retrospective clinical study. Ann Rehabil Med 2017;41(6):1065–1075.
27. Weissleder H, Weissleder R. Lymphedema: evaluation of qualitative and quantitative lymphoscintigraphy in 238 patients. Radiology 1988;167(3):729–735.
28. Kleinhans E, Baumeister RG, Hahn D, Siuda S, Büll U, Moser E. Evaluation of transport kinetics in lymphoscintigraphy: follow-up study in patients with transplanted lymphatic vessels. Eur J Nucl Med 1985;10(7-8):349–352.
29. Gloviczki P, Calcagno D, Schirger A, et al. Noninvasive evaluation of the swollen extremity: experiences with 190 lymphoscintigraphic examinations. J Vasc Surg 1989;9(5):683–689; discussion 690.