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Original Article

Three-Dimensional Imaging of Lymphatic System in Lymphedema Legs Using Interstitial Computed Tomography-lymphography

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As a new trial, we used interstitial computed tomography-lymphography (CT-LG) in 10 patients with lower extremity lymphedema (n = 20 limbs) at stage 0, 1, 2, or 3 under the International Society of Lymphology (ISL) classification. In all cases, CT-LG, lymphoscintigraphy, and indocyanine green fluorescence-lymphography (ICG-LG) were performed. In the examination of the ascending level of depicted lymphatic vessels, we measured the diameters of lymphatic vessels detected with CT-LG and conducted an image analysis of dermal backflow of lymph (DB). CT-LG had better resolution than lymphoscintigraphy and enabled the clear visualization of lymphatic vessels with a minimum lumen size of 0.7 mm. CT-LG also showed the three-dimensional architecture of the DB, which originated from deep lymphatic collectors via branched small lymphatic vessels. Our findings are quite valuable not only for detailed examinations of lymphedematous sites and for the lymphedema surgery, but also for investigations of the pathogenesis of lymphedema which has not yet been established. We observed that lymphoscintigraphy could show the lymphatic vessels up to the thigh level in all cases, whereas CT-LG enabled the vessels' visualization up to the leg level at maximum. In conclusion, CT-LG provided adequate and detailed three-dimensional imaging of the lymphatic system in lymphedema patients.

Key words: interstitial lymphography, CT lymphography, lymphedema, lympatic imaging

Secondary lymphedema is a chronic swelling of a limb that develops following lymph node dissection or radiation therapy for conditions such as an intrapelvic tumor, breast cancer, and melanoma. Major limb swelling reduces motor function, and the frequent occurrence of cellulitis leads to lowered activities of daily living (ADLs) in cancer survivors. Lymphedema is an abnormal congestion of the lymphatic system due to decreased lymphatic function. The pathophysiology of lymphedema is complex and not yet established [1–6]. The reason for this insufficient understanding of secondary lymphedema is a lack of anatomical information of the lymphatic system, especially the abnormal lymphatic system in lymphedema patients. Difficulties in live imaging of the lymphatic system also remain.

Therefore, in recent years, various methods for mapping the lymphatic vessels have been developed. There are pros and cons to all of these methods, and there is no established technique that visualizes thin and transparent lymphatic vessels in the living body. Several mapping methods are in use for lymphatic vessels in clinical settings.

In the direct contrast method, cannulation is directly performed on lymphatic vessels under micros-

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copy, and a contrast agent is injected. This method has been used since the 1950s [7], but it is invasive and there is a high risk of retention of contrast agent and possibly a pulmonary embolism; thus, the direct contrast method is not used frequently now.

The indirect contrast method, in which the contrast agent is subcutaneously or intradermally injected and then physiologically taken up by lymphatic vessels, is being developed in various ways because of its low invasiveness. Since 1953, lymphoscintigraphy has been used as the gold standard [8] for lymphatic examinations. Lymphoscintigraphy is less invasive and is able to capture the overall image of the lymphatic drainage pattern, but its weakness is the low definition of the resultant images. As lymphoscintigraphy requires a scintillation camera, there is also an issue with radiation exposure.

Indocyanine green fluorescence lymphography (ICG-LG) has become widespread since 2006 [9], when the near-infrared observation camera became commercially available. ICG-LG enables very detailed analyses of the network of lymphatic vessels without providing radiation exposure, and it makes dynamic observations possible. However, there are disadvantages of ICG-LG examinations as well, such as difficulty observing depths of ≥ 15 mm and difficulty in capturing the entire image.

MRI lymphography (MRL-LG) is a technique reported by Dimakakos *et al.* in 2007 [10]. Relatively high-definition images can be obtained without radiation exposure, but an MRL-LG examination requires approx. an hour of imaging time, meaning the image can easily become blurred. In addition, the contrast agent used for MRI can easily enter the venous circulation [11].

Computed tomography lymphography (CT-LG) was first reported by Suga *et al.* in 2003 [12]. Extremely high-definition images can be obtained, and the examination takes only about 5 min. Images can be obtained within a short time, and the imaging devices are relatively widely distributed. The main disadvantage of CT-LG is the radiation exposure.

We used the interstitial CT-LG method described by Suga *et al.* [13-15] to three-dimensionally and comprehensively observe the lymphatic vessels of the extremities in high definition using multi detector-row CT (MDCT). We used the gold standard (*i.e.*, lymphoscintigraphy) and ICG-LG, both of which are now widespread, on the same patients, and we compared the utility of CT-LG in lymphedema. This study is the first report regarding the use of CT-LG on extremities of lymphedema patients.

Table 1 Patient characteristics

age-years	mean ± SD	50±16
primary or secondray-no./total no. (%)		
	primary	6/20 (30)
	secondry	14/20 (70)
sex-no./total no (%)		
	male	2/20 (10)
	female	18/20 (90)
ISL stages-no./total no. (%)		
	stage 0	5/20 (25)
	stage 1	3/20 (15)
	early stage 2	5/20 (25)
	late stage 2	5/20 (25)
	stage 3	2/20 (10)

SD = standard deviation

Patients and Methods

Patient enrollment and physical examinations. The study subjects were 10 patients with lower extremity lymphedema (20 limbs) who underwent CT-LG, lymphoscintigraphy, and ICG-LG between November 2013 and August 2015 at Okayama University Hospital. Seven patients had secondary lymphedema (14 limbs), and 3 had primary lymphedema (6 limbs) (Table 1).

A blood test and chest X-ray examination were per-

Table 2 ISL staging of lymphedema

ISL stage	Features
0	A subclinical state. No oedema but presence of lymphatic impairment.
1	Mild oedema that is reversible with appropriate limb elevation.
2	Moderate oedema that is not reversible with limb elevation. Pitting present, except in late stage 2 when more fibrosis occurs.
3	Lymphostatic elephantiasis with trophic skin changes such as acanthosis, deposition of fat and fibrosis, warty overgrowth.

Sources: [19,20]

formed during the first examination, and patients with diseases other than lymphedema and those who were suspected of causing lower extremity edema were excluded. Two experienced lymphedema therapists recorded the patients' physical findings and symptoms, and edema stages were determined according to the International Society of Lymphology lymphedema stage classification (Table 2).

All studies were performed under the approval of the Ethics Committee of Okayama University Hospital (ethics No. 1052). In addition, after being provided with sufficient explanation, all patients provided informed consent to undergo the examinations and have their results published.

CT-LG. MDCT-LG was performed as described by Suga *et al.* [12] in 2003 to identify sentinel lymph nodes in breast cancer. An MDCT scanner (Aquilion TSX-101A; Toshiba Medical Systems, Tochigi, Japan) was used in this study. A total of 0.8 ml of 1% Xylocaine® (AstraZeneca, Osaka, Japan) was injected subcutaneously into the 1st to 4th interdigital web space of the dorsum of the foot, using a 30-gauge needle to reduce the pain. A total of 4 ml of Iopamidol (Iopamiron 370; Bayer Yakuhin, Osaka, Japan) was then equally injected intradermally using a 24-gauge needle at the exact same locations. After the iopamidol was administered, the injection sites ware massaged gently for 10 min to facilitate the migration of the contrast agent to draining lymphatics. Thirty min after the administration of iopamidol, contiguous 1-mm-thick CT images were acquired from tip of the foot to the groin area. The CT scanning was operated at 120 kV and 250 mA, with a 50-cm field of view and a 512×512 matrix. Three-dimensional CT images were then reconstructed using maximum intensity projection (MIP) and surface rendering techniques at a medical imaging analysis work station (AZE Virtual Place; AZE Corp., Tokyo, Japan).

Lymphoscintigraphy. The lymphoscintigraphic studies were performed by subcutaneously injecting 185 MBq ^{99m}Tc-DTPA-HSA (Techne®Albumin kit, FujiFilm RI Pharma, Tokyo, Japan), divided in the 1st and 4th interdigital web spaces. A total of 0.2 ml of the tracer was injected with a 27-gauge needle. Subsequently, the injected areas were massaged for 5 min and entire- body images were taken with a scinticamera (GE Discovery NM/CT670, GE Healthcare Japan, Tokyo, Japan) 5 and 30 min after the injection of the radioactive tracer [16].

ICG-LG. For the ICG-LG, 25 mg of ICG (DIAGNO-GREEN®, Daiichi-Sankyo, Tokyo) was dissolved in 2 ml of distilled water, and 2 ml of 1% Xylocaine® (AstraZeneca, Osaka, Japan) was added to reduce pain. A total of 0.2 ml of the solution was subcutaneously injected into the 1st and 4th interdigital web spaces. Subsequently, the patient was asked to walk for 30 min, and the lymphatic vessels were recorded with a Photo Dynamic Eye® (Hamamatsu Photonics, Shizuoka, Japan). Still images were captured from the video record [17].

Analysis of examination protocols and comparison of results. First, we evaluated the examination protocols. For the total of 20 limbs, we used early-stage lymphedema (stage 0, 5 limbs; stage 1, 3 limbs; early stage 2, 5 limbs) to determine the depiction rate of each part where lymphatic vessels were successfully imaged. We divided the depiction level in 4 groups: (1) static, (2) up to the dorsum of the foot, (3) up to the leg, and (4) up to the thigh. The results of lymphoscintigraphy, classified into the same 4 groups, were compared for the same limb. Then, for the 20 limbs, the diameters of 45 lymphatic vessels detected with CT-LG were measured in a perpendicular slice of lymphatic vessel, and the mean, minimum, and maximum values were calculated. The diameter was measured at the thinnest part of each lymphatic vessel detected.

Last, we performed an image analysis of the dermal backflow of lymph (DB) detected with CT-LG and performed an anatomic evaluation.

Results

Examination of the test protocols. The limbs with early-stage lymphedema (stages 0–early stage 2) were imaged well with lymphoscintigraphy, up to the thigh. However, when the same group was imaged with the present CT-LG protocol, the visualization of the transport of the contrast agent to the thigh was clearly poorer, including the following results: one limb with no movement from the injection site, 2 limbs that were imaged up to the dorsum of the foot, 10 limbs that were imaged up to the leg, and no limbs that were imaged up to the thigh level (Fig. 1).

The dose of the contrast agent for the CT-LG and that for the lymphoscintigraphy was 4 and 0.2 ml, respectively. A total of 4 injection sites were required for CT-LG (2nd and 3rd interdigital web spaces, in

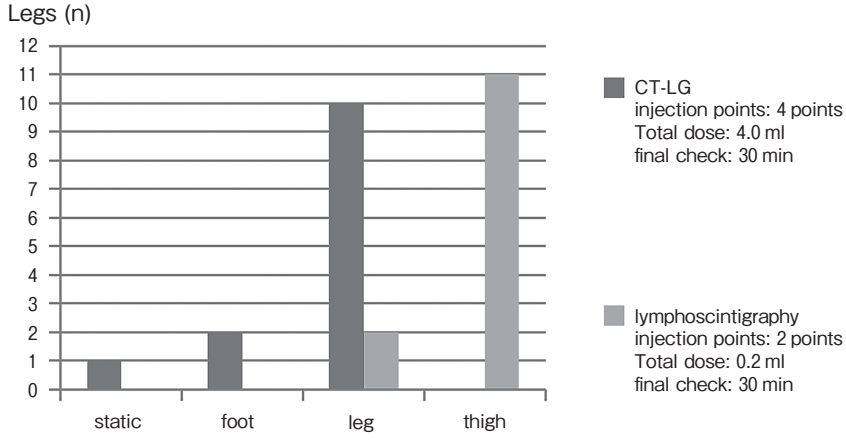

Fig. 1 Comparison of sites reached by the contrast agent. Lymphoscintigraphy (light gray bar) provided good imagery up to the thigh in almost all cases. However, CT-LG (dark gray bar) did not visualize as far as the thigh; in most cases it provided visualization only up to the leg. Only one case was static, in which the contrast agent was not taken up by lymphatic vessels.

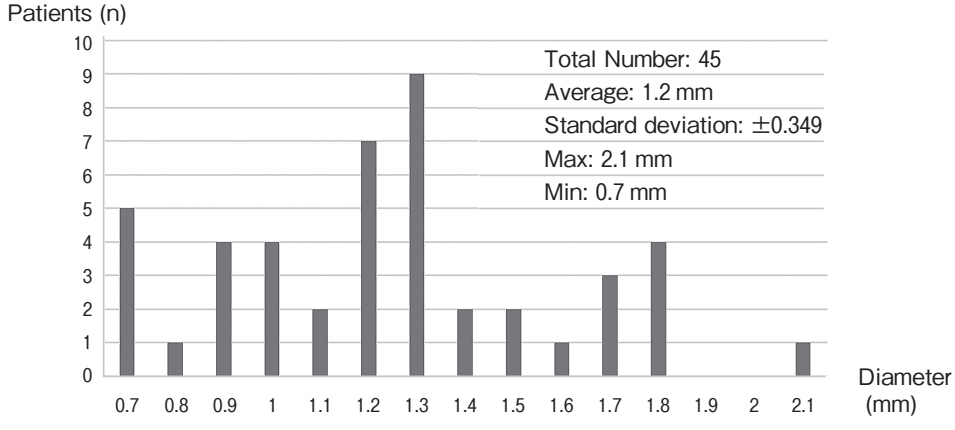

Fig. 2 Diameters of lymphatic vessels as observed with CT-LG. We were able to identity a total of 45 lymphatic vessels, and the mean diameter of the narrowest portion was 1.2 mm. The smallest lymphatic vessel identified was 0.7-mm-dia.

addition to the 1st and 4th used for lymphoscintigraphy). The final observation time was 30 min for both CT-LG and lymphoscintigraphy.

Anatomical evaluation of visualization of lymphatic vessels and DB with CT-LG. Our results demonstrated that CT-LG could depict the collecting lymphatic vessels, traveling in the deep layer to the superficial fascia in a tortuous manner, ascending toward the proximal part, and diverging to the several small lymphatics.

The mean and minimum diameters of the lumen of the lymphatic vessels imaged by CT-LG (n=45) were 1.2 and 0.7 mm, respectively (Figs. 2, 3). By CT-LG, the DB was imaged as an aggregation of high contrast at the dermal area, but unlike ICG-LG, DB types could not be confirmed.

Lymphatic vessels were confirmed underneath the DB, and the point at which the contrast agent flowed back from collecting lymphatic vessels to the dermis was observed three-dimensionally. The collecting lymphatic vessels under the superficial fascia branched into several small lymphatic vessels, and they traveled to the surface of dermis and then connected to the DB (Fig. 4). Lymphoscintigraphy and ICG-LG from the same location showed that only the DB, and lymphatic vessels underneath the DB were masked and not shown (Fig. 5).

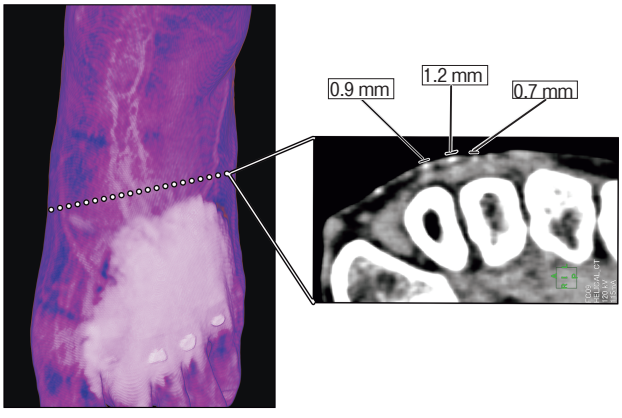

Fig. 3 Three-dimensional imaging of lymphatic system. In the images (left: 3D reconstructed image, right: 1-mm-slice image), lymphatic vessels were clearly identified with higher contrast than surrounding tissues, and the diameters were measured.

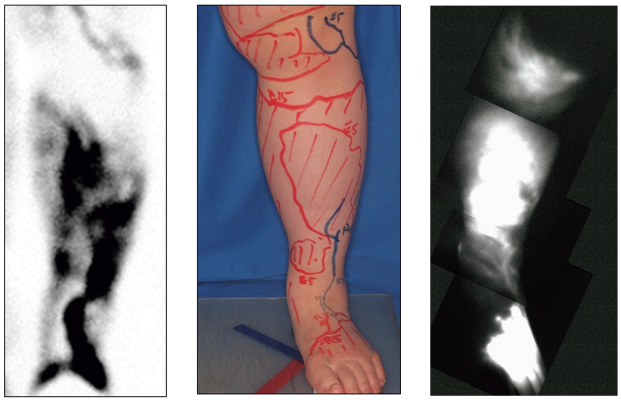

Fig. 5 Comparison of lymphoscintigraphy and ICG-LG. The same position as that shown in Fig. 3 is compared using lymphoscintigraphy (left) and ICG-LG (right). With lymphoscintigraphy, the proximal part is visualized better than with CT-LG but the resolution is low and two-dimensional; therefore, bundles of lymphatic vessels were observed as a lymphatic pathway, but individual vessels could not be observed in detail, and a detailed observation of DB parts could not be made. DB spread was also detected by ICG-LG, and as it is a two-dimensional image, the three-dimensional structures deep under the DB cannot be analyzed.

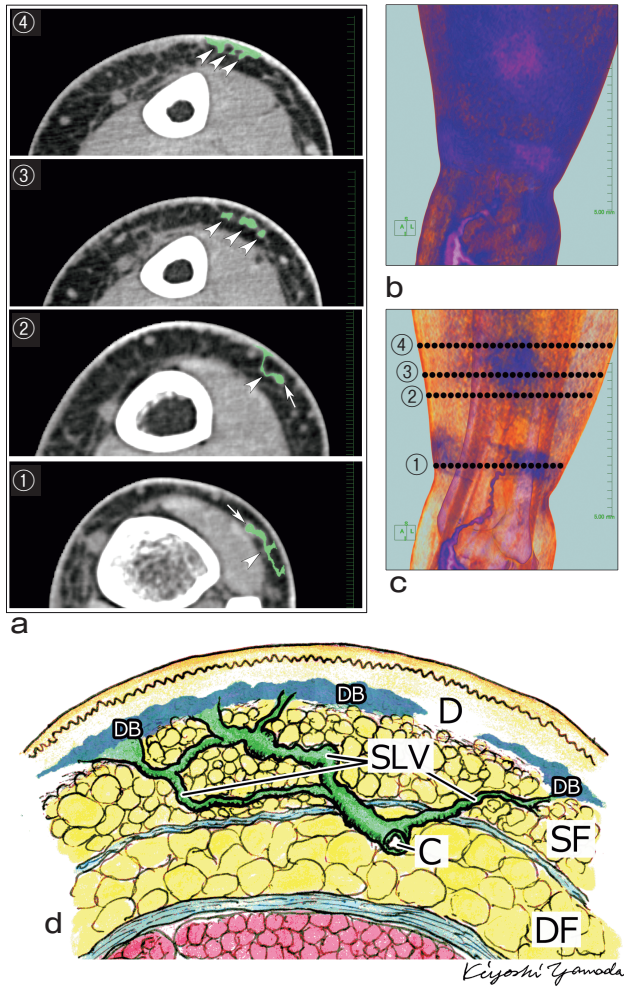

Fig. 4 Detailed analysis of DB sites with CT-LG. Slice images (**a**, 1-4) show some collecting lymphatic vessels (arrow) branching upward and medially toward the dermis (arrowhead), transitioning to the DB. In the representative high-contrast 3D image (**b**), lymphatic vessels were hidden under DB and could not be observed, but in the representative lower-contrast 3D image (**c**), lymphatic vessels that were previously hidden under the DB were observed. Panel **d** is a schematic representation of the DB. C, Collector; SLV, small lymphatic vessel; D, dermis; SF, superficial fat layer; DF, deep fat layer; DB (blue area in the dermis), dermal backflow.

Discussion

In the present study, we used CT-LG, a method widely used for the imaging of sentinel lymph nodes, in patients with lower-extremity lymphedema. The protocol for this study was determined and implemented based on the protocol for sentinel lymph node testing. For comparison, we also performed lymphoscintigraphy, the gold-standard examination of the lymphatic system, and ICG-LG, which has become popular in recent years, for the same patients.

To verify the protocol, we compared the usefulness of CT-LG with that of lymphoscintigraphy in early-stage lymphedema patients showing less degeneration of the

lymphatic system. With lymphoscintigraphy, lymphatic vessels were well visualized up to the thigh. With CT-LG by the present protocol in same group, the uptake of the contrast agent was limited to the dorsum of the foot and the leg, and the depicting results for the thigh were poor.

Previous reports of the CT-LG protocol for sentinel node examination in breast cancer revealed good visualization of the lymphatic vessels [12-15], and in the present study peripheral lymphatic vessels were mostly well depicted by CT-LG. Thus, there was no problem with uptake by lymphatic vessels in CT-LG. The reason for poor visualization of lymphatic vessels at thigh level in CT-LG was probably the slower flow of the contrast agent in lymphatic vessels, or alternatively the uptake of the contrast agent may be less in the lymphatic system compared to the radio-isotope tracer used in lymphoscintigraphy. Further improvement in the CT-LG protocol is needed. Considering the fact that lymph flows centrally over time, increasing the imaging time to 30 min or more and increasing the exercise load in addition to massaging the injected sites to increase the lymphatic flow [9, 17] may also be helpful.

We anatomically evaluated CT-LG with the well-imaged dorsum of the foot and leg. Lymphatic vessels of typical diameter for collecting lymphatics (*i.e.*, 0.7-2.1 mm) were observed at high resolution. In lymphoscintigraphy, bundles of lymphatic vessels were observed as a lymphatic pathway, but individual vessels could not be observed in detail, which is a major advantage of CT-LG. In addition, lymphoscintigraphy and ICG-LG are two-dimensional examinations, and thus information from deeper tissues cannot be obtained. Therefore, CT-LG with its three-dimensional observation of deeper tissues provides new information. There are reports of ICG-LG with efficient observation of DB types and classification, but such classification was not possible with CT-LG. In other words, in terms of resolution, CT-LG is inferior to ICG-LG. This could be improved with the development of future CT devices.

Though the type of DB could not be confirmed in the present study, the 3D CT-LG images offer fine high resolution; we were thus able to confirm images indicating the mechanism of DB. From collecting lymphatic vessels, several thinner lymphatic vessels branch out toward the dermis, transitioning to the DB. To the best of our knowledge, we are the first to report three-dimensional findings of this anatomy. The term 'DB' was coined because lymph accumulates in either skin or subcutaneous tissues macroscopically and/or physically [18], but it was unknown where lymph leaked from and how it flowed back to accumulate in the dermis. According to the information obtained in the present investigation, lymphatic vessels that branch from collecting lymphatic vessels toward the dermis have an inner diameter wide enough to be confirmed with CT. This was likely the observation of lymph flowing back to new or existing abnormal lymphatic vessels as it moves toward the dermis. From there, lymph flows back through capillary lymphatic vessels, leading to its storage in interstitial spaces (Fig. 4). It is unclear why such a phenomenon occurs there, but due to upstream blockage or increased internal pressure of lymphatic vessels, lymph appears to flow back from deeper areas to shallower areas, as if to escape.

In the present CT-LG protocol, imaging at the thigh level was poor, but the resolution of the anatomical information on the imaged areas was superior to that provided by lymphoscintigraphy, and the information of the deep layer was far better than shown by both lymphoscintigraphy and ICG-LG. These characteristics of CT-LG could help to reveal the lymph flow in lymph-circulation disorders, and the anatomical information provided by CT-LG would be a great advantage to perform conservative or surgical treatment for lymphedema patients. When the location of the DB and the developmental mechanism are accurately visualized, this information would be valuable when selecting surgical sites in the currently used lymphatico-venous anastomosis (LVA) procedure for creating a lymph drainage route in lymphedema patients.

The main limitation of the present study is that CT could only be performed once, to avoid multiple exposures to radiation. Therefore, the protocol did not allow for the imaging of central body parts. Thus, in the future, it is necessary to investigate the optimum timing while exploratory adjusting the time from the administration of the contrast agent to the imaging of the CT, and a protocol suitable for routine examinations should be established.

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References

1. Mortimer PS, Bates DO, Brassington HD, Stanton AWB, Strachan DP and Levick JR: The prevalence of arm oedema following treatment for breast cancer. QJM (1996) 89: 377-380.
2. Schrenk P, Reiger R, Shamiyeh A and Wayand W: Morbidity following sentinel lymph node biopsy versus axillary lymph node dissection for patients with breast carcinoma. Cancer (2000) 88: 608-614.
3. Ozaslan C and Kuru B: Lymphoedema after treatment of breast cancer. Am J Surg (2004) 187: 69-72.
4. Sakorafas GH, Peros G, Cataliotti L and Vlastos G: Lymphedema following axillary lymph node dissection for breast cancer. Surg Oncol (2006) 15: 153-165.
5. Hong JH, Tsai CS, Lai CH, Chang TC, Wang CC, Lee SP, Tseng CJ and Hsueh S: Postoperative low pelvic irradiation for stage I-IIA cervical cancer patients with risk factors other than pelvic lymph node metastasis. Int J Radiat Oncol Biol Phys (2002) 53: 1284-1290.
6. Ryan M, Stainton MC, Slaytor EK, Jaconelli C, Watts S and Mackenzie P: Aetiology and prevalence of lower limb lymphoedema following treatment for gynaecological cancer. Aust N Z J Obstet Gynaecol (2003) 43: 148-151.
7. Kinmonth JB: Lymphangiography in man; a method of outlining lymphatic trunks at operation. Clin Sci (Lond) (1952) 11: 13-20.
8. Sherman A and Ter-Pogossian M: Lymph-node concentration of radioactive colloidal gold following interstitial injection. Cancer (1953) 6: 1238-1240.
9. Unno N, Inuzuka K, Suzuki M, Yamamoto N, Sagara D, Nishiyama M and Konno H: Preliminary experience with a novel fluorescence lymphography using indocyanine green in patients with secondary lymphedema. J Vasc Surg (2007) 45: 1016-1021.
10. Lohrmann C, Foeldi E and Langer M: Indirect magnetic resonance lymphangiography in patients with lymphedema preliminary results in humans. Eur J Radiol (2006) 59: 401-406.
11. Liu NF, Lu Q, Jiang ZH, Wang CG and Zhou JG: Anatomic and functional evaluation of the lymphatics and lymph nodes in diagnosis of lymphatic circulation disorders with contrast magnetic resonance lymphangiography. J Vasc Surg (2009) 49: 980-987.
12. Suga K, Ogasawara N, Okada M and Matsunaga N: Interstitial CT lymphography-guided localization of breast sentinel lymph node: preliminary results. Surgery (2003) 133: 170-179.
13. Suga K, Yuan Y, Ueda K, Kaneda Y, Kawakami Y, Zaki M and Matsunaga N: Computed tomography lymphography with intrapulmonary injection of iopamidol for sentinel lymph node localization. Invest Radiol (2004) 39: 313-324.
14. Suga K, Yuan Y, Okada M, Matsunaga N, Tangoku A, Yamamoto S and Oka M: Breast sentinel lymph node mapping at CT lymphography with iopamidol: preliminary experience. Radiology (2004) 230: 543-552.
15. Yamamoto S, Suga K, Maeda K, Maeda N, Yoshimura K and Oka M: Breast sentinel lymph node navigation with three-dimensional computed tomography-lymphography: a 12-year study. Breast Cancer (2016) 23: 456-462.
16. Maegawa J, Mikami T, Yamamoto Y, Satake T and Kobayashi S: Types of lymphoscintigraphy and indications for lymphaticovenous anastomosis. Microsurgery (2010) 30: 437-442.
17. Shinaoka A, Yamada K and Kimata Y: Indocyanine green fluorescent lymphography and microsurgical lymphaticovenous anastomosis; in ICG fluorescence imaging and navigation surgery, Kusano M, Kokudo N, Toi M, Kaibori M eds, 1st Ed, Springer Japan, Tokyo (2016) pp 433-441.
18. Sty JR, Boedecker RA, Scanlon GT and Babbitt DP: Radionuclide "dermal backflow" in lymphatic obstruction. J Nucl Med (1979) 20: 905-906.
19. International Society of Lymphology: The diagnosis and treatment of peripheral lymphedema. Consensus document of the International Society of Lymphology. Lymphology (2003) 36: 84-91.
20. Chiu TW: Management of secondary lymphoedema. Hong Kong Med J (2014) 20: 519-528.