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Quantitative Lymph Imaging for Assessment of Lymph Function using Indocyanine Green Fluorescence Lymphography

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Abstract *Objectives:* A new diagnostic imaging technique that can assess lymph function is needed as a screening test in daily practice. This study assessed the use of indocyanine green (ICG) fluorescence lymphography in subjects without leg oedema.
Methods: 0.3 ml of ICG (0.5 %) was injected subcutaneously at the dorsum of the foot. Subsequently, the movement of ICG dye from the injection site to the groin was traced by visualizing its fluorescence signal with an infrared light camera. The time for the dye to reach the knee and groin were measured (Transit time to knee: TT_K, Transit time to groin: TT_G). TT_G was measured while standing, lying at a supine position, standing with massage, and sitting while using a cycle ergometer exercise at an intensity of 50 W at 50 rpm in ten healthy volunteers at intervals of 14 days.
Results: Mean TT_G during standing was 357 ± 289 and 653 ± 564 seconds for the right and left legs respectively. Compared to TT_G in the standing position, all other conditions shortened TT_G. In another seventeen subjects without leg oedema, we compared transit time obtained with ICG fluorescence lymphography to that with dynamic lymphoscintigraphy. A significant correlation between transit time measured with ICG lymphography and dynamic lymphoscintigraphy was identified ($r^2 = 0.64$, $p < 0.01$).
Conclusions: ICG fluorescence lymphography has the potential to become an alternative lymphatic imaging technique to assess lymph function.

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Introduction

Lymph flow imaging was reported first with direct lymphography using oil contrast agents[1,2] and later with lymphoscintigraphy using radioisotopes.[3–5] Although direct lymphography provides highly accurate and informative images of lymph flow, the test is seldom performed at present because it is a tedious procedure to expose foot lymphatics and there is a risk of complications from the contrast agent.[6–8] Currently, lymphoscintigraphy is considered a major imaging modality for the diagnosis of patients with lymphoedema and for evaluating lymphatic disorder in the swollen extremity.[9,10] Lymphoscintigraphy can detect retarded tracer transport even in mild lymphoedema without morphological abnormalities and is useful to evaluate the functional lymph flow in patients with lymphatic reconstruction.[11] Quantitative analysis of lymph transport is performed by obtaining dynamic images or measuring the transit time for injected dye to reach the groin.[12] However, lymphoscintigraphy is time consuming, expensive, and potentially teratogenic during pregnancy, so that the technique has only been performed on selected patients in clinical practice.[8]

Recently, we introduced a new technique to observe the lymph flow to visualize fluorescence of near-infrared light of indocyanine green (ICG) as a tracer for the diagnosis of lymphoedema.[13] In that study, we reported several characteristic images of fluorescence in patients with secondary lymphoedema such as dermal back flow, obstructed fluorescence images with dilatation, which was compatible with the lymposcintigraphic findings.[13] The technique is safe and minimally invasive. Furthermore, the technique allows real time video images to be observed by tracing the ICG dye movement together with patients on the monitor of a laptop computer. ICG has been widely used in a variety of clinical situations such as examination of hepatic function, and retinal angiography with minor side effects.

In this study, we describe ICG fluorescence lymphography as a new method for quantitatively assessing lymph function.

Methods

Measurement of transit time in ICG fluorescence lymphography

Ten healthy volunteers (all men) between the ages of 24 and 48 (mean 33.1 $\pm$ 7.9 years) took part in this study, who had no past history of cardiovascular disorders. Prior to the study, duplex scanning was performed to confirm the absence of venous insufficiency or deep vein thrombosis (DVT) in the participants.

0.3 ml of indocyanine green (ICG: Diagnogreen 0.5%; Daiichi Pharmatical, Tokyo, Japan) was subcutaneously injected at the dorsum of the foot with a 27-gauge needle. Immediately after the injection, fluorescence images of subcutaneous lymphatic drainage were obtained using an infrared camera system (PDE™; Hamamatsu Photonics K.K. Hamamatsu, Japan), which activates ICG with emitted light (wavelength:760 nm) and filters out light with a wavelength below 820 nm. The light source for emission of ICG consisted of 760-nm LEDs, and the detector was a charge-coupled device (CCD) camera. The fluorescence images were continuously observed on the monitor of a laptop computer (LaVie G, Type T; NEC Co., Tokyo, Japan). After injection of ICG at the dorsum of the foot, fluorescent images of ICG dye were traced (Fig. 1), and the interval until the dye reached the groin was measured (Transit time). The transit time was measured under the following four conditions in each individual: standing, lying at a supine position, standing with massage, and sitting while performing cycle ergometer exercise at an intensity of 50 W at 50 rpm. Because the fluorescence signals at the dorsum of the foot remained for at least 7 days, repeated measurements of transit time under each condition were performed at intervals of at least 14 days.

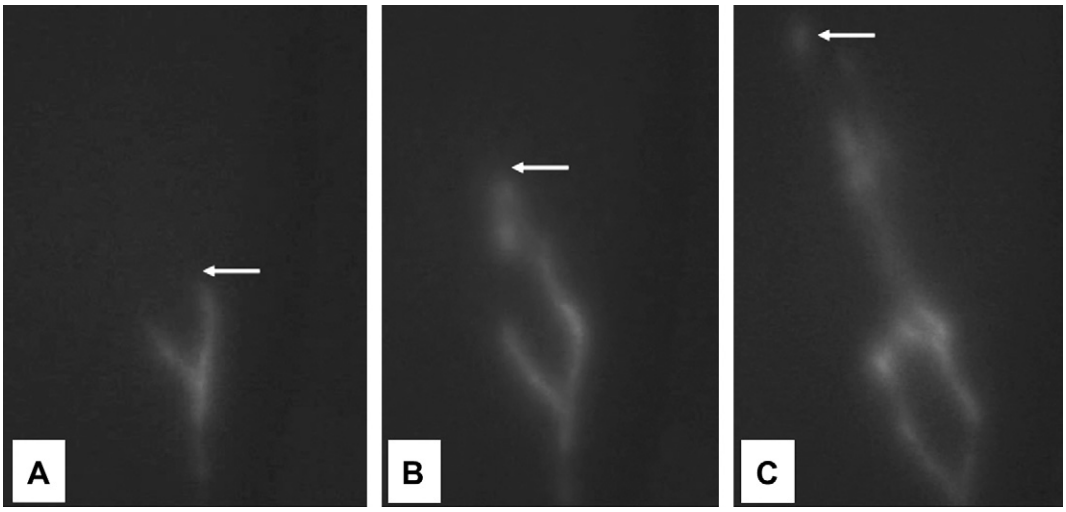

Figure 1 ICG fluorescence lymphography: real time Images of superficial lymph vessel (A): Fluorescence of ICG obtained in lymph vessel (B): ICG dye pushed forward to the proximal region in lymph propulsion (C): ICG dye further pushed forward. Arrows indicate the most advancing ICG dye in lymph vessel.

Comparison of transit time between dynamic lymphoscintigraphy and ICG fluorescence lymphography

Seventeen male patients (mean age 74.7 ± 7.5 years) with an abdominal aortic aneurysm who were hospitalized in our surgical ward agreed to undergo an investigation of their lymphatic function and underwent both lymphoscintigraphy and ICG fluorescence lymphography before abdominal aortic aneurysm (AAA) surgery. Duplex scanning was also performed in these patients to exclude venous insufficiency or deep vein thrombosis.

ICG fluorescence lymphography was also performed in a supine position as described above. With real time observation on the monitor of the laptop computer, both Transit time (TT) from the dorsum to the knee (TT_K) and TT from the dorsum to the groin (TT_G) were measured (Fig. 2). On the next day, 0.3 ml of technetium 99m-labeled human serum albumin diethylenetriamine pentaacetic acid (^{99m}Tc-HSA-D, activity 111 MBq) (Nihon Medi-Physics Co., Ltd., Nishinomiya, Japan) was injected subcutaneously at the dorsum of the foot at a distance of 1 cm from the site at which ICG was previously injected. The subject remained supine for imaging of the leg. Sequential images including both the knee and inguinal regions were obtained every 30 seconds for a period of 20 minutes using a gamma camera, Dual-Head Variable-Geometry Nuclear Imaging System (Millennium VG, GE Healthcare, Chalfont St Giles, United Kingdom), and time—activity curves of the knee and groin were created. The counts per second of each region were plotted against time. The times at which the counts rose were defined as the lymphatic transit time to the knee (TT_K) and groin (TT_G), respectively.

All data were expressed as mean ± standad deviation, and the differences in the means between the groups were assessed using paired one-sample Student's *t* test. Regression, correlation was calculated by GrapPad Prism (ver.5, GraphPad Software, CA, USA).

Results

Transit time in ICG fluorescence lymphography in different positions

Fluorescence images of continuous lymph channels running along the medial aspect of the leg from the injection site to the groin were traced with a handy camera probe. Lymph propulsion was observed with pulsatile lymph flow images, which suggested the pump function of lymphatic vessels (Fig. 1). With ICG fluorescence lymphography, TT_G at a standing position was 357 ± 289 and 653 ± 564 seconds for the right and left legs respectively. Among the

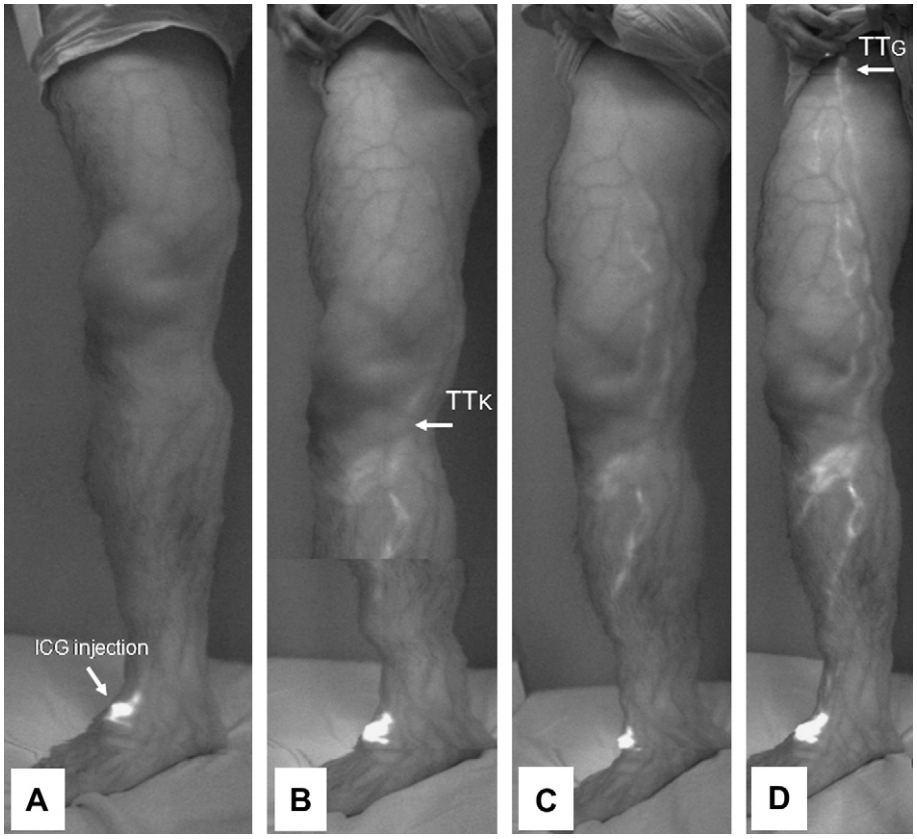

Figure 2 Panoramic views of ICG fluorescence lymphography in a healthy volunteer: Sequential transition of ICG dye in lymph vessel. (A): Immediately after injection of ICG at the dorsum of foot. Arrow indicates the injection site. (B): Transit time to the knee (TT_K) was measured when ICG dye reached the knee (arrow). (C): ICG dye was further propelled toward the groin. (D): Transit time to groin (TT_G) was measured when ICG dye reached the groin (arrow).

participants, values differed ten-fold. The supine position significantly shortened TT_G on both sides compared to that in standing position (157.3 ± 107.7, 279.0 ± 189.4 seconds, right leg ($P = 0.030$), left leg ($P = 0.029$), respectively, (Fig. 3A). Standing with massage also significantly shortened TT_G bilaterally (53.9 ± 16.2, 60.3 ± 34.9 seconds, right leg ($P = 0.009$), left leg ($P = 0.007$), respectively, (Fig. 3B). Moreover, TT_G in a sitting position while exercise using bicycle ergometer also seemed to be shorter than that in a standing position (183.4 ± 93.2, 237.3 ± 177.2 seconds, right leg ($P = 0.094$), left leg ($P = 0.047$), respectively, (Fig. 3C).

Comparison of transit time between dynamic lymphoscintigraphy and ICG fluorescence lymphography

In 17 subjects, both dynamic lymphography and ICG fluorescence lymphography were performed with an interval of 24 hours to measure both TT_K and TT_G in each individual. With ICG fluorescence lymphography, TT_K and TT_G in a supine position in 17 subjects were 203.6 ± 166.9 and 329.6 ± 311.5 seconds, respectively. With dynamic lymphoscintigraphy, TT_K and TT_G were measured from the time-activity curves (Fig. 4B.C). Gamma camera showed the sequence of the ^{99m}Tc- HSA-D movement from the dorsum of the foot to the groin via the knee (Fig. 4A). TT_K and TT_G in a supine position were 264.7 ± 276.3 and 481.8 ± 407.1 seconds, respectively. There was a strong correlation between transit time measured with ICG lymphography and dynamic lymphoscintigraphy (Fig. 5).

Discussion

We previously reported the usefulness of fluorescence ICG lymphography to facilitate diagnosis of secondary lymphoedema and described the morphological characteristics of fluorescence lymph imaging. ICG lymphography clearly visualized the dilated, tortuous lymph vessels with proximal obstruction as well as dermal backflow. ICG absorbs light in

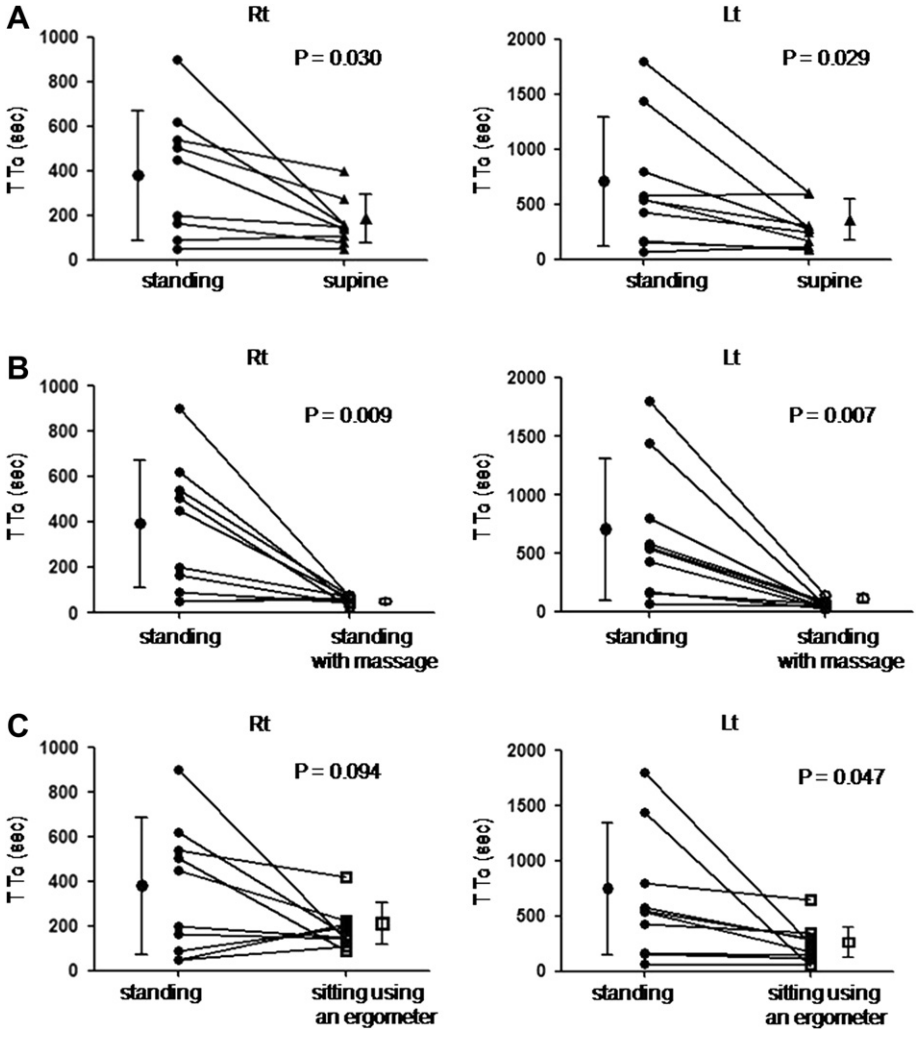

Figure 3 Transit time to groin (TT_G) in ICG fluorescence lymphography in different positions. Data from individual participants were plotted for each leg in each condition. The mean (± S.D.) of TT_G was also shown. (A) Standing position vs supine position (B) Standing position vs standing with manual massage (C) Standing position vs sitting with exercise using ergometer.

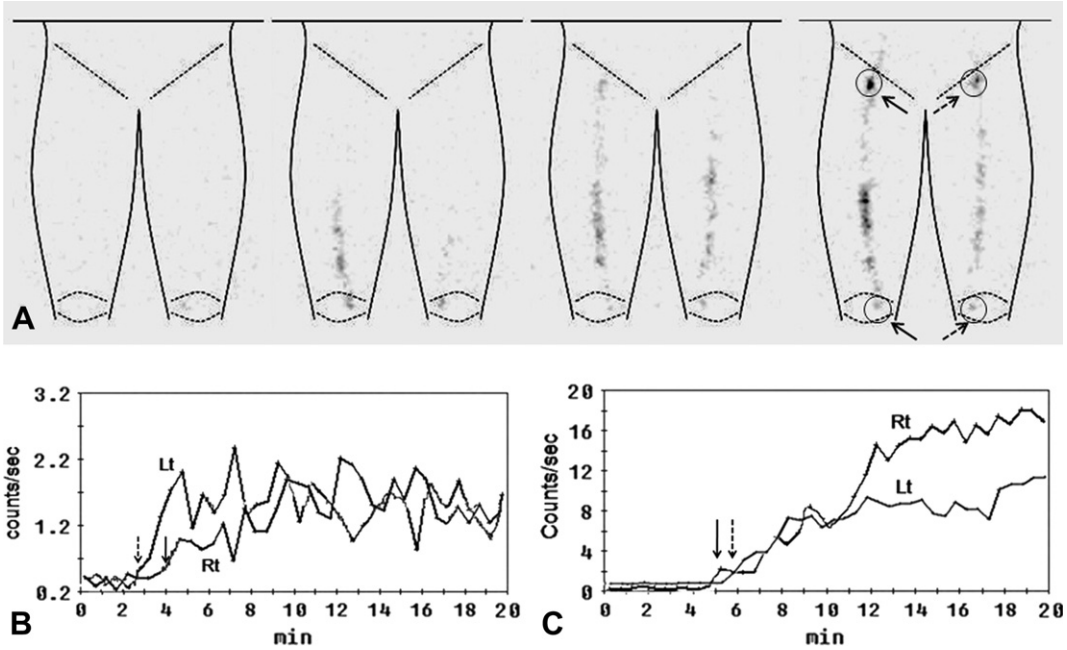

Figure 4 (A) Dynamic lymphoscintigraph of the lower limb. Circle and arrows indicate the site of knee and groin where the time-activity curves were created. Solid arrow: right side. dashed arrow: left side. (B) Bilateral time-activity curves at knee. Arrows indicate the transit time to knee (TT_K) (C) Bilateral time-activity curves at groin. Arrows indicate the transit time to groin (TT_G).

the near-infrared range, with a maximum at 805 nm. The excitation wavelength of ICG that produces the maximum fluorescence is 765 nm. ICG fluorescences with a maximum at 840 nm in plasma.[14] The fluorescence of ICG in the near infrared wavelength can deeply penetrate living tissue and is advantageous for obtaining visual information. The greatest advantages of this imaging technique include its ease and real time visualization as well as safety. Recently, Ogata *et al*. applied the technique intraoperatively to assist lymphaticovenular anastomoses for patients with lymphoedema.[15] In this study, we also took advantage of the real-time visualization of ICG lymphography and succeeded in measuring transit time (i.e. interval until appearance at the region of interest) in subjects without leg oedema. Because the infrared camera clearly visualized lymph propelled toward the proximal region, it was easy to measure the time from the injection site to the region of interest, although the camera limits the detection of lymphatic vessels located less than 2 cm from the body surface so that the detection of deep lymph vessels, which run in the subfascial space, may be difficult with this system, particularly, in a fat subject or a fat-rich region. In this study, pulsatile lymph flow was identified in a fluorescent vessel in a supine position, which was compatible with the notion proposed by Smith *et al*. that spontaneous contractions are an important means by which lymph is propelled.[16] Sharma *et al*. measured lymph velocities as 0.23—0.75 cm/s by determining the transit time of ICG fluorescence signal traversing 2—16 cm length of surface lymph vessels in swine.[17] In this study, the transit time in a supine position was shorter than that in a standing position. Like the venous system, the standing position creates hydrostatic pressure so that the lymph trunk must propel lymph against gravity. With massage or exercise using an ergometer, the transit time was also shortened, indicating that muscle contraction could accelerate lymph flow and/or pump pressure. Many other studies have confirmed the role of muscular contraction or limb exercise in promoting lymph flow, which is similar to leg venous flow propelled by a ''muscle pump''.[18,19] However, our study scale was too small and further study is needed to confirm the results. In this study, the ethical committee in our institute did not approve the performance of radioisotope scintigram in healthy volunteers who are relatively young. Therefore, we performed the comparison of transit times between ICG lymphography and dynamic lymphoscintigraphy in preoperative AAA patients without venous diseases who comprised a relatively older population. Because the

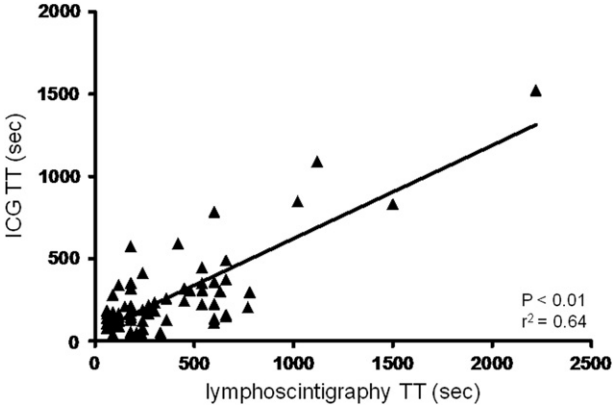

Figure 5 Linear regression analysis shows a comparison of transit time measured by dynamic lymphoscintigraphy vs ICG fluorescence lymphography.

preoperative patients were hospitalized in our surgical ward under similar conditions, uniform injection of both ICG and radioisotope was performed at exactly the same time within a 24-hour interval. There was a significant correlation in transit time between both tracers. However, there is a possibility that subcutaneous injection with a 1 cm distance and 24-hour interval may affect tracer uptake and change the transit time due to local inflammatory response. Therefore, simultaneous injection of both ICG and radioisotope might result in even better correlation between the tracers' transit times.

The transit time, measured in relation to the tracer distance, was introduced in dynamic scintigraph.[20,21] The transport capacity of lymph is determined not by the speed of lymph flow but by the volume over time, transit time is definitely not the sole parameter of lymph function. Nonetheless, many previous studies measured transit time as one of parameters to assess lymph function, or at least included transit time among the categories of evaluating lymph function.[12,22] Previous reports[23,24] identified that the measurement of transit time, and combination of qualitative and quantitative assessment with lymphoscintigraphy, enhance the diagnostic sensitivity for detecting lymphoedema particularly for patients with mild or incipient symptoms. Ohtake *et al*. reported that transit time to the groin ranged between 2 and 6 minutes, and Mandel *et al*. reported the time to be around 10 minutes in healthy subjects without leg oedema.[20,25] In our study, TT_G in a supine position was between 2 and 5 minutes with ICG lymphography and between 3 and 5 minutes with dynamic lymphoscintigraphy, which was similar to the values reported in previous studies. These results, together with the significant correlations between transit time measured with ICG lymphography and dynamic lymphoscintigraphy, suggest ICG lymphography may become an alternative method of assessing lymph function. Currently, none of the quantitative lymphoscintigraphy has been standardized for application in daily practice because many hospitals use various radioisotopes at different radioactivity doses under different protocols of image interpretation. Either intradermal or subcutaneous injection also affects the results.[24,26] Consequently, quantitative lymphoscintigraphy have been performed only for selected patients with swollen extremities in a limited number of hospitals, where not only an expensive gamma camera system is available but also a well-trained technician together with radiologists. As described above, our technique is reliable only for the study of superficial lymphatics ($<$ 2 cm to the skin). Therefore, the importance of deep lymphatic chains cannot be assessed with this method. However, in healthy individuals or lymphoedema patients with incipient symptoms, the superficial lymphatics are maintained and may play a greater role in lymph drainage than deep lymphatic chains. Our method may detect the early stages of malfunction of superficial lymphatics before irreversible damage is identified as morphological changes on lymphography.

In this study, we performed ICG fluorescence lymphography and measured transit time only for subjects with normal legs. Although the results were encouraging as a new diagnostic method to assess lymph function, further study is needed to apply those technique to patients with swollen extremities to identify differences from images obtained from healthy volunteers. Moreover, other causes of leg oedema such as venous insufficiency, cirrhosis, and malnutrition should also be evaluated with this method to determine whether these causes affect the lymph function or not. With application of quantitative analysis of lymph function together with qualitative assessment to such patients with leg oedema, we may detect lymphatic disorder concurrent with other diseases.

In conclusion, ICG fluorescence lymphography is safe and minimally invasive, and can be performed repeatedly. Measurement of the transit time for lymph flow to the region of interest using this technique may facilitate the assessment of lymph function in patients with swollen legs.

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