Using indocyanine green fluorescent lymphography to demonstrate lymphatic architecture

Hiroo Suami, David Chang, Roman Skoracki, Kiyoshi Yamada, Yoshihiro Kimata

Abstract

Background: Visualisation of the lymphatic system is a challenging task. Recently, an indocyanine green (ICG) fluorescent lymphography system was developed for visualising the lymphatic vessels. ICG emits energy in the near-infrared region between 840 and 850 nm when it is bound to protein in the tissue. *Aim:* To use ICG fluorescent lymphography to identify locations of the lymphatic vessel for lymphovenous shunt. *Methods:* The lymphatic anatomy in the upper extremity was investigated using ICG fluorescent lymphography in 3 healthy volunteers and 15 patients with breast cancer-related lymphoedema prior to them undergoing lymphaticovenular bypass. *Results:* In healthy volunteers, fluorescent images of lymphatic vessels emerged at the dorsal hand as a shiny linear pattern and ran longitudinally towards the proximal arm after a few minutes. In lymphoedema patients, the lymphatic vessels could be identified at the dorsal hand, but the appearance of the lymphatic structure varied between patients. *Conclusions:* ICG fluorescent lymphography allows for the prompt identification of the lymphatic vessels and has the potential to improve the outcomes of lymphovenous shunt operations and for use as a diagnostic tool. *Declaration of interest:* None.

Key words

Breast cancer-related lymphoedema
Indocyanine green fluorescent lymphography
Lymphaticovenular shunt operation
Near-infrared radiation

Visualisation of the lymphatic system is a challenging task. The collecting lymphatic vessels are small in size, with diameters of between 0.2 and 1.0 mm, lymphatic fluid is colourless, and our knowledge of lymphatic system anatomy is uncertain. Lymphatic vessels contain numerous valves at short intervals. They regulate lymphatic flow from distal to proximal, and, therefore, lymphatic tracers have to be injected in the distal sites.

The current mainstay of lymphatic imaging is lymphoscintigraphy using radionuclide tracers, which has become a standard preoperative imaging study for breast and skin cancer patients to help detect the sentinel lymph node/s to which the lymphatic vessels from the primary cancer site drain (Morton et al, 1992; Giuliano et al, 1997; Uren et al, 1999). However, this procedure needs to be performed in a radiology room and the low-resolution 2-dimensional images are not suitable for demonstrating fine lymphatic capillaries.

Recently, an indocyanine green (ICG) fluorescent lymphography system was developed for visualising the lymphatic vessels (Giuliano et al, 1997; Kitai et al, 2005; Murawa et al, 2007; Ogata et al, 2007; Unno et al, 2007; Tsujino et al, 2009). Indocyanine green (ICG) is a water-soluble compound that has been widely used for assessing cardiac output, hepatic function, and ophthalmic angiography for more than half a century. ICG contains sodium iodide and should be used with caution in patients with a history of iodide allergy.

Although previous diagnostic examinations used its absorption spectra, ICG also emits energy in the near-infrared region between 750–810 nm when bound to protein in the tissue (Benson and Kues, 1978). This feature is advantageous for investigating deep-tissue structures because near-infrared radiation can penetrate tissue without the interference suffered by visible-wavelength radiation, which can be absorbed by haemoglobin and water.

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Methods

An ICG fluorescent lymphangiography system (Photodynamic Eye; Hamamatsu Photonics, Japan) was used in this study. The device is composed of a camera unit, which includes a black and white charge-coupled device camera for recording video images, near-infrared emitting diodes, and a controller unit that operates the camera (***Figure 1***).

A laptop computer (Latitude E6400; Dell, USA) was used for monitoring video images and recording them in digital format. The charge-coupled device camera has a fixed focus ranging from 15–25 cm, which allows investigation of a 10×10 cm^2 field with one image. The system can detect anatomical structures by detecting near-infrared radiation in the tissue at a depth of about 10 mm from the skin surface.

Lymphography of healthy volunteers

Lymphatic anatomy in the upper extremity was investigated using ICG fluorescent lymphography in three healthy volunteers who had no medical history of vascular disease or lymphoedema. Some 0.01–0.02 mL of ICG (0.025–0.05 mg) was injected into each finger web intradermally. After the injection, the volunteers clasped and unclasped their hands several times. The arm was scanned and the lymphatic pathways were marked on the skin with a skin marker.

Lymphography of breast cancer patients with lymphoedema

From March 2010–March 2012, the authors, using a clinical protocol approved by the institutional review board of the University of Texas MD Anderson Cancer Center, Houston, undertook ICG fluorescent lymphography in 34 patients with breast cancer-related unilateral lymphoedema. Patients were under general anaesthesia and the ICG lymphography was performed prior to a lymphaticovenular shunt operation in order to detect locations of the collecting lymphatic vessels.

ICG was injected intradermally into each finger web, as well as at two or three locations at the volar side of wrist. The arm was massaged manually to facilitate uptake into the lymphatics. Identified lymphatic pathways were marked on the skin.

Figure 1. (Left) Intraoperative scene showing the use of incocyanine green (ICG) fluorescent lymphography. (right) The ICG fluorescent lymphography system with a laptop computer.

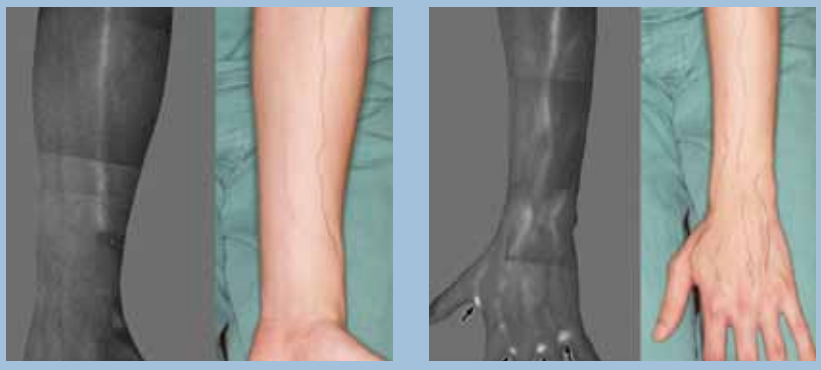

Figure 2. Incocyanine green fluorescent lymphography images (black and white, montage from video images) of the healthy upper limb, with photos of the limb (colour).

Results

In the healthy volunteers, the fluorescent markers in the lymphatic vessels emerged at the dorsal hand as a shiny linear pattern and ran longitudinally towards the proximal arm a few minutes post-injection (***Figure 2***). They ascended in the posterior forearm and then gradually changed direction towards the medial side of the upper arm, en route to the axilla. Each lymphatic vessel was independent, and there were few interconnections between them. The flow was facilitated by spontaneous muscle movement and squeezing the vessel from the outside.

In lymphoedema patients, the mean age was 54.5 ± 8.5 years; mean BMI 29.3±5.6 kg/m^2; and mean per cent volume increase 29.3 ± 20.9. The lymphatic vessels could be identified at the dorsal hand. However, appearances of the lymphatic structure varied in each patient.

Some patients showed a clear, linear pattern similar to the healthy volunteers showed only one or two blurred linear lines (***Figure 3***). In the latter cases, a patchy reticular honeycomb-like structure appeared after 10–15 minutes. The vessels included in this patch were sharper and smaller than the lymphatic vessels demonstrated in the healthy volunteers. This suggested that these vessels were located in more superficial layers and they were considered lymphatic capillaries in the dermal layer.

This reticular structure could be seen in the forearm and upper arm, but the size of the area was different in each lymphoedema patient. Skin incisions of 2 cm were made at sites where linear patterns were marked, and prominent and patent lymphatic vessels were found. Surgical findings and image data correlated with high accuracy.

Discussion

Hudack and McMaster (1933) reported the first attempt to identify the lymphatic

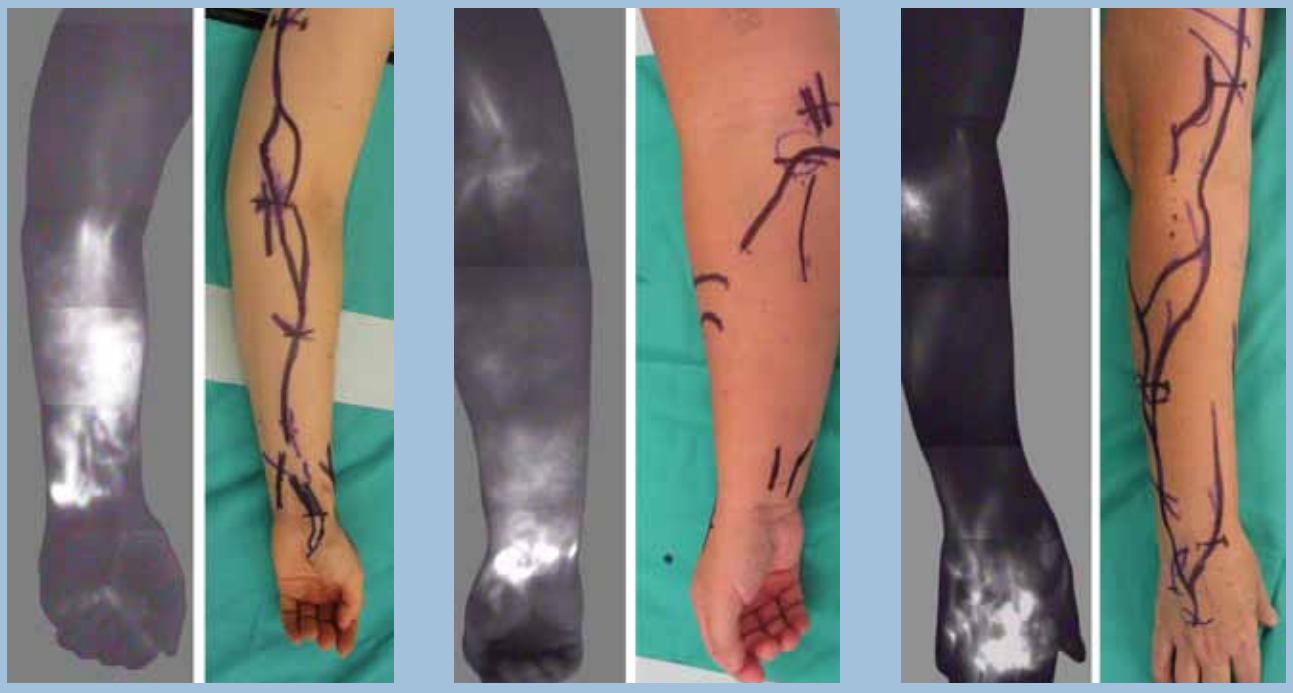

Figure 3. Incocyanine green (ICG) fluorescent lymphography image of the dorsal (left) and volar (centre) sides of a lymphoedema limb; injection sites of ICG and identified collecting lymphatic vessels. (Right) Preoperative view of the patient. Ink marks indicate the positions of the collecting lymphatic vessels found during the surgery.

system in a living human. They injected dye into the skin in their forearms to visualise the lymph capillary.

Kinmonth (1952) used the same dye and injected it into patients to identify the collecting lymphatic vessel in the dorsal side of the hand and foot. Kinmonth was not only able to identify the collecting lymphatic vessel, but also cannulated under a microscope, and injected radiocontrast media directly into the lymphatic vessel. This radiographic examination is called lymphography or lymphangiography. This work lead to a better understanding of lymphatic pathways. However, lymphangiography is gradually being phased out of clinical practice because of cumbersome procedures, lengthy examination time with slow radiocontrast injection, and the possibility of causing pulmonary embolism.

Lymphoscintigraphy is more straightforward than lymphangiogram, but is mainly used for detecting lymph nodes rather than lymphatic vessels.

The authors' initial aim of using ICG fluorescent lymphography was to identify the locations of the lymphatic vessel for lymphovenous shunt allowing skin incisions can be made precisely over the collecting lymphatic vessels. This method for the prompt identification of the functional lymphatic vessels also has the potential to significantly improve the outcomes of lymphovenous shunt operations.

The authors demonstrated the differences in the anatomy of the lymphatic system between healthy upper limbs and lymphoedematous limbs. Reticular structures were only observed in the lymphoedematous limbs.

These findings are similar to the 'dermal backflow' sign observed in lymphangiography and lymphoscintigraphy (El-Kharadly and Enein, 1965; Feldman et al, 1966; Sty et al, 1979). This sign has also been investigated anatomically using human cadaver specimens and amputated limbs (Crockett, 1965; Suami et al, 2007a; 2007b). These studies showed that dermal backflow is caused by incompetent valves in precollectors following obstruction of the superficial collecting lymphatic vessels (***Figure 4***).

ICG fluorescent lymphography can be performed without any special preparation or radiation. Thus, this system may become a standard tool for functionally assessing lymphoedema and could also help establish a new algorithm for lymphoedema treatment.

Yamamoto et al (2011) investigated lymphatic architectural changes in asymptomatic lower extremities of patients who underwent gynecological and urological cancer treatment using the ICG lymphography. They could detect dermal backflow sign in asymptomatic limbs and concluded that this would be an early sign of lymphoedema in latent phase.

Recently, sentinel node biopsy using ICG fluorescent lymphography has been reported in breast and skin cancers (Kitai et al, 2005; Murawa et al, 2007; Tsujino et al, 2009). Another feasible application would be in individuals with skin cancer involving the scalp, because lymphatic maps of the scalp are highly variable and the collecting lymphatic vessels run superficially (Uren et al, 1999; Pan et al, 2008). Also, the ICG fluorescent lymphography may be used during axillary dissection to preserve arm lymphatic pathways such as the reverse

axillary mapping concept (Nos et al, 2007; Thompson et al, 2007).

Conclusion

ICG fluorescent lymphography was used in this study to compare lymphatic structures in normal and lymphoedematous upper limbs. The system was useful in locating functional collecting lymphatic vessels in a lymphoedematous arm in which the normal lymphatic structures had been substantially altered. The use of ICG fluorescent lymphography may improve outcomes following lymphovenous shunt operations. This technology has the potential to be used for various applications in the future. JL

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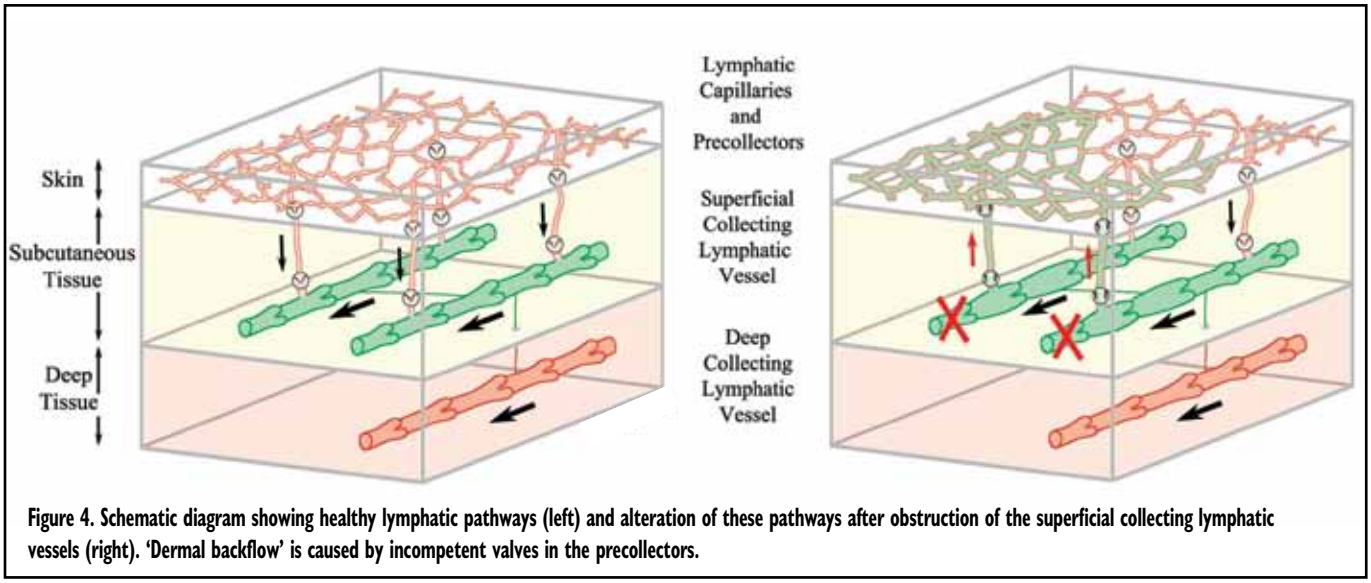

Figure 4. Schematic diagram showing healthy lymphatic pathways (left) and alteration of these pathways after obstruction of the superficial collecting lymphatic vessels (right). 'Dermal backflow' is caused by incompetent valves in the precollectors.