Near-Infrared Fluorescence Lymphatic Imaging in Lymphangiomatosis

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Abstract

Background: Lymphangiomatosis is a rare disorder of the lymphatic system that can impact the dermis, soft tissue, bone, and viscera and can be characterized by lymphangiomas, swelling, and chylous discharge. Whether disordered lymphangiogenesis in lymphangiomatosis affects the function and anatomy of the entire systemic lymphatic circulation or is localized to specific sites is not fully known.

Methods and Results: A 35-year-old Caucasian female diagnosed with whole-body lymphangiomatosis at 2 months of age and who continues to present with progressive disease was imaged with near-infrared fluorescence lymphatic imaging. While the peripheral lymphatics in the extremities appeared largely normal compared to prior studies, we observed tortuous lymphatic vessels, fluorescence drainage from the peripheral lymphatics into lymphangiomas, and extensive dermal lymphatics in the left thigh and inguinal regions where the subject had previously had surgical assaults, potentially indicating defective systemic lymphangiogenesis.

Conclusions: Further research into anatomical and functional lymphatic changes associated with the progression and treatment of lymphangiomatosis could aid in understanding the pathophysiology of the disease as well as point to treatment strategies.

Introduction

LYMPHANGIOMATOSIS IS A RARE LYMPHATIC DISORDER in which multiple lymphangiomas, abnormal lymphatic cystic structures, develop within the patient.¹ Lymphangiomas can form anywhere lymphatic tissue is present, are commonly categorized in part by their depth and size, and generally fall into one of three broad types: (i) lymphangioma circumscriptum, which encompasses lymphangiomas with cutaneous vesicles that frequently have a blistery or warty appearance; (ii) cavernous lymphangiomas, which typically occur deeper in the dermis but do not involve the outer skin and are typically one to several centimeters in diameter; and (iii) cystic lymphangiomas, which generally occur in the loose connective tissues and can grow very large if no intervention is taken.^{2,3} It is not uncommon for patients with lymphangiomatosis to have multiple types of lymphangiomas and across multiple organs and tissue sites,⁴ suggesting a generalized and systemic defect in the process of lymphangiogenesis.

The disease can present at any time during life although it generally manifests prior to adulthood.⁴ Although lymphangiomas are typically benign, significant morbidities can

occur when they invade the lungs, skeleton, or other critical organs and/or compress adjacent structures.² The etiology of lymphangioma is poorly understood, but is thought to be a result of congenital defects in the formation of the lymphatic system in which lymphatic cisterns become detached from or fail to connect to the normal lymphatics.^{5,6} Treatment options are limited and typically include diet, drainage or surgical removal, sclerotherapy, radiation therapy, and/or laser treatment.^{2,7,8} Unfortunately, owing to the difficulty in identifying and destroying all the abnormal lymphatics associated with a lymphangioma, there tends to be a high rate of recurrence.⁴

Herein, we report the case of a patient with a history of lymphangiomatosis who presented to our clinic for imaging of the peripheral lymphatics using investigational, near-infrared fluorescence lymphatic imaging (NIRFLI). We sought to determine whether the peripheral lymphatics were altered in anatomy and function.

Subject and Methods

This study was conducted under US Food and Drug Administration (IND#: 102,827) and University of Texas Health

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Science Center at Houston Institutional Review Board approval and in accordance with the Helsinki Declaration of 1975, as revised in 2008.

The subject was a 35-year-old Caucasian female who was diagnosed with whole-body lymphangiomatosis at 2 months of age and, as detailed in Table 1, had an extensive medical history related to the disease including two lung surgeries, multiple debulking procedures, left and right vulvectomies including bilateral inguinofemoral lymphadenectomy, as well as a history of ascites, chylous ascites, and pleural effusion. Reports from truncal CT scans 5 months prior to presentation in our clinic indicated the presence of lymphangiomas and pockets of fluid throughout the thoracic, abdominal, and pelvic regions. Figure 1A–1C shows images of the subject's arms and legs just prior to imaging, as well as images of the lymphangiomas observed in the left pelvic region and the extensive scarring in the left leg from previous tissue expansion and debulking procedures. The subject had no history or evidence of lymphedema (Fig. 1A).

The subject presented at our clinic for NIRFLI, an investigational technique which enables the noninvasive visualization of peripheral lymphatic architecture and contractile function following the intradermal administration of indocyanine green (ICG). NIRF lymphatic imaging has been previously reported, and distinct differences between healthy and diseased lymphatics, such as in lymphedema, have been noted.^{9–12} To maximize the opportunity to visualize the lymphatics in the subject we opted to focus our NIRF lymphatic imaging primarily on one leg and one arm, with a secondary focus on the abdomen and the contralateral leg. Because the subject reported that her left leg and right arm were more symptomatic than their corresponding contralateral limbs, we selected those limbs as our primary focus for imaging.

Following informed consent, a total of sixteen injections of 25 μ g of ICG in 0.1 mL of saline were administered. Initially we administered six injections in the left leg, and four injections in the right arm, as shown in Figure 1D. After imaging the leg and arm, four additional injections were administered in the abdomen near the navel, and two final injections were administered the right leg, with each set of injections followed by imaging. Immediately after injection, images were acquired for 2 hours by illuminating the limbs with the diffuse output of a 785 nm laser diode to excite the ICG laden lymph. A custom, intensified charge coupled device (ICCD) camera was then used to collect the resultant fluorescent signal (830 nm) emanating from the lymphatics. Exposure times of 200 ms allowed the acquisition of movies of contractile lymphatic function.

Images were analyzed to determine the presence of lymphatic abnormalities such as dermal backflow, characterized by dense networks of fluorescent lymphatic capillaries and/or extravascular fluorescence, as well as dilated and/or tortuous lymphatic vessels. The movement of fluorescent lymph through the lymphatics was also noted and, as described previously,¹¹ its contractile propulsion was measured by calculating the average propulsion velocity of packets of fluorescent lymph propagating between two regions of interest along the lymphatic vessel. The period between propulsion events was determined by measuring the time lapse between two successive packets of fluorescent lymph propagating through a single region of interest. The subject was contacted 24 hours after imaging to verify that no adverse events occurred. The results of imaging were not used in clinical care. Unfortunately, as a consequence of her disease, the subject expired several months after imaging.

As shown in Figure 2, the lymphatics in the lower left leg were generally well defined with active lymphatic propulsion (see Supplementary Video 1; supplementary material is available online at www.liebertpub.com/lrb) although

TABLE 1. MEDICAL HISTORY OF SUBJECT

<i>Year of Life</i>	<i>Diagnosis and/or procedures</i>
1 st	Lymphangiomatosis
4 th	Right lung surgery
7 th	Left lung surgery
10 th –11 th	Abdominal debulking and reconstruction including a tissue expander located in left thigh
11 th	Vulvectomy, complete radical left, with inguinofemoral lymphadenectomy; Left thigh debulking (first of four procedures)
14 th	Depression; Dysmenorrhea (with a presumptive diagnosis of endometriosis)
16 th	Left thigh debulking (last of four procedures)
21 st	Osteoarthritis of spine; Degenerative joint disease of the spine; Back surgery; Lumbar discectomy; Paraesthesia of left lower extremity
27 th	Tachycardia; Chylous ascites; Spontaneous bacterial peritonitis (first of six occurrences)
33 rd	Vulvectomy, complete radical right, with inguinofemoral lymphadenectomy; Thrombosis; Embolism including pulmonary embolism; Warfarin -induced coagulopathy; Pneumonia
34 th	Ascites; Transaminitis; Pulmonary embolism; Gastrointestinal bleed from colonic ulcer; Peritonitis (last of six occurrences)
36 th	Partial small bowel obstruction; Abdominal pain; Acute liver failure; Acute mastitis; Hypertension; Headache; Encephalopathy; Deep vein thrombosis; Plural effusion; Death

Results

As shown in Figure 2, the lymphatics in the lower left leg were generally well defined with active lymphatic propulsion (see Supplementary Video 1; supplementary material is available online at www.liebertpub.com/lrb) although

FIG. 1. The clinical appearance of the patient's (A) extremities show no evidence of lymphedema, (B) left pelvic region with superficial lymphangiomas, and (C) scarring in the left medial thigh. (D) Schematic illustrating the location of the intradermal injections of indocyanine green. The injections on the left leg and right arm were administered first, then after the initial imaging, the injections in the abdomen and right leg were administered. Total dose of ICG was 400 micrograms.

tortuous lymphatics were observed in the lateral and medial calf. Furthermore, the peripheral lymphatics in the upper left leg manifested extensive dermal lymphatics that actively propelled fluorescent lymph from the medial thigh towards the lateral thigh (Supplementary Video 2). In addition,

packets of lymph are clearly observed being propelled through a larger and deeper lymphatic vessel from the injection site in the left thigh towards the inguinal region despite the fact that the vessel itself is not clearly delineated beneath the fluorescent dermal lymphatics.

FIG. 2. Montages of the (A) ventral left leg with inset image of the lateral calf and (B) medial lymphatics in the left leg. See Supplementary Video 1 for movie of lymphatic contractile flow in the linear lymphatics in the lower left leg, and Supplementary Video 2 for a movie showing the movement of dye within the dermal lymphatics in the medial thigh.

FIG. 3. (A) Montage of fluorescent images illustrating abnormal crosstalk between the lymphatics in the inguinal regions, and (B) fluorescent image of the lateral left hip. The bright spots in both (A) and (B) correspond to the lymphangiomas visible in Figure 1B.

The presence of larger functional lymphatics away from the medial thigh in conjunction with the active propulsion seen in the medial dermal lymphatics may explain why this subject had not developed any sign of lymphedema despite the repeated surgical trauma inflicted on the medial thigh. In

contrast, while fluorescent dermal lymphatics, commonly referred to as dermal backflow, are frequently seen in subjects with lymphedema, they typically have little observable propulsion and, more often than not, appear to bloom from, instead of delivering lymph to, the larger lymphatic vessels.^{10,11,13,14} No adverse events were observed during this study.

The fluorescent lymph in the left inguinal region drained into the right inguinal region, as shown in Figure 3A, and the lymphangiomas on both the left and right sides were fluorescent (Fig. 3B). The crosstalk between the left and right inguinal regions is unusual, although it is similar to the pelvic regions of some lymphedema subjects with genital or lower abdominal involvement,¹⁵ as well as a subject with Parkes-Weber syndrome who also had fluorescent lymphoceles on the groin.¹⁶

As shown in Figure 4, and with the one exception of a tortuous fluorescent lymphatic vessel connecting the injection sites on the medial wrist, the lymphatics in the right arm were linear and well-defined as typically seen in healthy subjects. Fluorescent nodes were also observed in the axilla (Fig. 4C).

The lymphatics in the abdomen above the navel were tortuous and drained towards the axillary regions, as shown in Figure 5. Dermal backflow was observed around the injection sites above the navel, indicating lymphatic communication across the midline of the subject. In addition, a tortuous lymphatic vessel, which was very faint, was observed actively propelling fluid from the left injection site towards the right axilla (Supplementary Video 3). The injections below the navel had no clear lymphatic drainage pathway towards the inguinal or axillary regions, although there was a fluorescent lymphatic vessel observed between them.

The lymphatics in the lower right leg (Fig. 6A) were linear and well-defined though the lymphatic vessel draining from the foot to the inguinal region was very faint (Fig. 6B), most likely owing to the fact that we only administered one injection on the foot and lower leg. The injection sites on the

FIG. 4. The lymphatics in the right arm with (A) the palm down, (B) the palm up, and (C) with the arm over the head.

FIG. 5. The lymphatics in the abdomen above the navel were tortuous and drained towards the axillary regions. Fluorescent lymphatic capillaries were also observed around the injection sites above the navel. The injections below the navel had no clear lymphatic drainage pathways.

right leg drained toward the right inguinal with no indication of dermal backflow in the thigh.

The average velocity in the well-defined lymphatics observed in the legs and arm was 0.9 cm/sec and 1.1 cm/sec, respectively, with an average period of 28 sec between propulsion events. These values are similar to those that we have measured previously in healthy subjects¹¹ and, in the case of the arm velocity, is similar to the fastest transit time reported by Yamamoto et al.¹⁷ The dermal lymphatics observed in the medial thigh also actively propelled lymph towards the lateral thigh (Supplementary Video 2) with an

average velocity of 1.4 cm/sec and an average period of 19 sec. The faster propulsion rates in these dermal lymphatics may enable these smaller, more superficial lymphatics to remove excess fluids adequately from the medial thigh, thus preventing the accumulation of interstitial fluids and proteins that are indicative of lymphedema and its associated sequelae.

While the subject reported multiple lymphangiomas throughout her body, the lymphatics in the right arm, lower left leg, and the abdomen largely appeared to be intact with contractile function. The lack of ICG pooling in those areas indicate that any abnormal lymphatics associated with local lymphangiomas are indeed disconnected from the normal lymphatics, as has previously been reported.^{6,18} The obvious exception to this are the extensive fluorescent dermal lymphatics observed in the upper left leg and bilateral inguinal regions, and the presence of fluorescence in the superficial lymphangiomas located in the inguinal region. However, these dermal lymphatics may not be a direct result of the lymphangiomas itself, but rather a result of significant lymphatic trauma associated with the subject's bilateral vulvectomies and inguinofemoral lymphadenectomies, as well as the placement of a tissue expander in the left thigh (Table 1).

One may speculate that the abnormal anatomy and function may be due to defects in lymphangiogenesis following surgical assault. Indeed, significant scarring is readily seen on the left medial thigh (Fig. 1C) where the extent of fluorescent dermal lymphatics is the greatest (Fig. 2B). Some, if not all, of the surviving lymphatics in the legs likely leak lymph directly into the inguinal basins where it is subsequently taken up by the other collecting lymphatics, including both the normal lymphatics and the abnormal lymphatics associated with the lymphangiomas.

Because the abnormal lymphatics are not thought to be directly associated with normal peripheral lymphatics, the ability to use NIRFLI to find and identify cavernous and/or cystic lymphangiomas may be limited, and conventional

FIG. 6. The lymphatics in the (A) lower right leg were linear and well-defined, although the lymphatic vessel draining from the foot to the inguinal region wrapped around the back of the calf and was very faint (B), most likely owing to the fact that we only administered one injection on the foot. The lymphatics draining the injection on the right thigh drained toward the right inguinal with no indication of dermal backflow.

imaging modalities such as MRI and ultrasound, which may not require exogenous contrast to discriminate fluid filled voids from surrounding tissues, may be needed. However, as shown herein, NIRFLI may provide a unique opportunity to visualize cross communication between the abnormal lymphatics associated with lymphangiomas and the normal lymphatics if/when it does occur.

In addition, if ICG were to be injected directly into a known lymphangioma, the fluorescent signal could potentially help to identify the extent of the diseased tissues and to plan and carry out appropriate therapeutic procedures. For example, as has been shown previously for sentinel lymph node dissection (for review, see Zhu and Sevik-Muraca¹⁹) and surgical guidance of lymphaticovenular anastomoses,^{20,21} NIRFLI may provide opportunities for surgical guidance in the resection of all the abnormal lymphatics associated with a lymphangioma, thereby reducing the chance for recurrence. This would be particularly useful in the resection of localized lymphangiomas that have a higher probability for cure if all the abnormal lymphatics are removed.

Conclusion

We have demonstrated the use of NIRFLI to image normal and abnormal lymphatics in a subject with lymphangiomatosis. While NIRFLI may be limited in its use to discover deep abnormal lymphangiomas, we demonstrate its potential use to discover communication between the lymphangiomas and normal peripheral lymphatics. Additional studies are needed to determine if NIRFLI could provide a needed tool to guide intervention to destroy abnormal lymphatics associated with known lymphangiomas and visualize changes that are associated with disease progression.

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Author Disclosure Statement

JCR and EMS own stock in a UTHealth portfolio company, NIRF Imaging, Inc., which seeks to commercialize the NIRF lymphatic imaging technology. JCR has also received consulting fees from NIRF Imaging, Inc.

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