Near-infrared fluorescence lymphatic imaging in a patient treated for venous occlusion

John C. Rasmussen, PhD, Melissa B. Aldrich, PhD, Renie Guilliod, MD, Caroline E. Fife, MD, Thomas F. O’Donnell Jr, MD, and Eva M. Sevick-Muraca, PhD, Houston and The Woodlands, Tex; and Boston, Mass

Although lower extremity edema/lymphedema can result from venous or lymphatic abnormalities, effective treatment depends on understanding their respective contributions to the condition. Herein we use near-infrared fluorescence lymphatic imaging in a 16-year-old girl diagnosed with unilateral lymphedema of the right leg and previously treated with left iliac vein stenting in an attempt to alleviate lymphedema. The imaging shows that abnormal lymphatic anatomy, rather than venous occlusion, was likely responsible for unilateral swelling. (J Vasc Surg Cases 2015;1:201-4.)

The venous and lymphatic systems are intimately linked through embryologic development and causation of edema. In patients with lower extremity edema, both systems may be dysfunctional. Yet, whereas venous abnormalities are often clinically obvious, lymphatic dysfunction is not routinely assessed. Lymphatic dysfunction has been demonstrated with bipedal lymphography in patients with Klippel-Trénaunay syndrome1 and with lymphoscintigraphy in patients with May-Thurner syndrome,2 suggesting a potential dual venous and lymphatic etiology for edema.

Understanding the underlying cause of edema is key to management. Although imaging is commonly employed to diagnose venous disease, lymphatic imaging is used less frequently. We present the case of an adolescent girl who underwent stenting of a left iliac stenosis for the indication of reducing lymphedema in the right leg. On referral to us for persistent edema, we used investigational near-infrared fluorescence lymphatic imaging (NIRFLI) to assess lymphatic drainage in both legs. This report is published with consent of the subject.

CASE REPORT

The subject was diagnosed at the age of 12 years with unilateral lymphedema praecox of the right leg. Phlebography revealed a 90% stenosis of the contralateral (left) iliac vein and abundant venous collaterals across the pelvis to the right iliac system (Fig 1, A). There was no previous hematoma or other causative event associated with her lymphedema. According to her records from another institution, it was hypothesized that the additional venous load produced by the collateral veins reduced the venous outflow on the right side, which contributed to her lymphedema. At the age of 14 years, her treating physician successfully stented the left iliac vein (Fig 1, B), but no clinical improvement was noted in her edematous right leg. At the age of 16 years, the subject presented to our clinic for lymphatic imaging as part of a Food and Drug Administration- (IND 102,827) and Institutional Review Board-approved investigational study for off-label use of indocyanine green (ICG) using NIRFLI (NCT00833599; www.clinicaltrials.gov). At presentation, the subject was 5.5 feet tall with a body mass index of 20.7 and a 34% increase in volume of the right leg over the left (Fig 2, A and B). After informed consent of the subject and her guardian, 12 injections, each containing 25 μg of ICG in 0.1 mL of saline, were intradermally administered, as shown in Fig 2, C, for uptake into the lymphatic plexus for mapping of reported lymphatic drainage pathways.3 NIRFLI was accomplished by illuminating the legs with diffuse 785-nm light and collecting the resultant 830-nm fluorescent signal emanating from ICG-laden lymph with a customized camera using night vision technology (for review of NIRFLI and ICG lymphography, see reference 4). Images were evaluated for abnormal dermal backflow (in which lymphatic vessels drain distally into the lymphatic capillaries), tortuous lymphatic vessels, and lymphatic contractile dysfunction.5,6

Distinct differences in lymphatic anatomy and contractile function were observed between left and right legs, as illustrated in Fig 3 and in Supplementary Videos 1 and 2. Whereas tortuous lymphatic vessels and extensive dermal backflow were observed in the lymphedematous (right) leg, the lymphatics in the asymptomatic (left) leg were linear and well defined, as typically seen in control subjects. Regular lymphatic contractile propulsion events,
associated with the lymph “pump,”7 were observed moving lymph toward the inguinal region in the asymptomatic leg (Supplementary Video 1). For each contractile event, the apparent velocity of a “packet” of ICG-laden lymph was computed along with the period of time between successive contractile events.5 In the subject’s asymptomatic leg, 61 contractile events were observed with an average propulsion velocity of 0.8 ± 0.2 cm/s and an average period of 76 ± 37 seconds. Whereas diffuse

Fig 1. Venograms of the left iliac occlusion and collateral veins before (A) and after (B) stent placement. Images obtained from the subject’s medical record.

Fig 2. Images of the upper (A) and lower (B) legs of the subject. (C) Location of the injection sites. Injection sites were covered with sterile bandages and, when the fluorescent signal oversaturated the camera, black vinyl tape.
movement of dye was observed in areas of dermal backflow in the right leg, only four contractile events, with an average propulsion velocity of 0.4 ± 0.1 cm/s and an average period of 57 ± 7 seconds, were observed in the vessel on the lateral ankle (Fig 3, A, inset, and Supplementary Video 2). Previously, we found the average velocity to be 0.9 ± 0.7 cm/s with a period of 52 ± 36 seconds in the legs of control subjects and 0.8 ± 0.4 cm/s and 72 ± 45 seconds and 0.8 ± 0.5 cm/s and 65.3 ± 46.4 seconds in symptomatic and asymptomatic legs of persons diagnosed with lymphedema, respectively. Thus, whereas the asymptomatic left leg possessed normal lymphatic anatomy, the period is similar to that seen in the symptomatic and asymptomatic legs of lymphedema patients. The average propulsion velocity in the symptomatic leg was half that observed previously, although the period was comparable to that of normal legs. No lymphatic pathways were observed traversing the pelvis between the left and right inguinal regions as previously observed in a subject with Parkes-Weber syndrome. To summarize, the lymphatic anatomy and function of this subject’s lymphedematous right leg and asymptomatic left leg are consistent with those observed in the lymphedematous and asymptomatic legs of subjects with lymphedema.

**DISCUSSION**

Most clinicians rely on both history and physical examination to diagnose lymphedema: for secondary lymphedema, a history of extirpative surgery or radiation therapy for neoplasm, major trauma, or recurrent cellulitis; whereas for primary lymphedema, female gender and left lower extremity edema beginning at menarche are suggestive of the diagnosis. Elephantine limb enlargement, dorsal “buffalo hump” over the metatarsals, and presence of Stemmer sign are associated with a lymphatic etiology. Although duplex ultrasound imaging is used routinely to define anatomy and venous function to objectively diagnose venous disease, there is no standard diagnostic for lymphedema. Lymphography, a surgical procedure requiring cannulation of a lymphatic vessel, provides only anatomic, but not functional, information. Lymphoscintigraphy, requiring intradermal or subcutaneous injection of a radionuclide, is based on the proximal movement of the tracer by lymphatic pumping function. Although it was not performed in this case, lymphoscintigraphy has been advocated as a method for the differential diagnosis of edema and has been used to confirm the clinical diagnosis of lymphedema. In a series of 188 patients, Cambria et al used lymphoscintigraphy to measure the transit time to regional lymph nodes and the appearance of lymph vessels and nodes to devise a modified Kleinhans transport index based on radionuclide distribution pattern. Using this approach, they accurately differentiated lymphatic causes of edema from venous and other causes.

NIRFLI uses soluble dye that provides immediate contrast as it is propelled with lymph, providing a more rapid, highly resolved, and nonradioactive assessment of lymphatic anatomy that has been previously demonstrated to more accurately detect lymphatic abnormalities than lymphoscintigraphy and enables visualization of local lymphatic transport functionality that cannot be directly determined by lymphoscintigraphy. Furthermore, by providing trace administration of ICG in several regions of the limb, a more comprehensive mapping of the limb-draining lymphatics can be obtained with a prognostic significance for the patient.

In this case, NIRFLI showed no anatomic abnormality but reduced propulsive lymph pumping on the asymptomatic leg compared with normal legs and both abnormal lymphatic anatomy and limited contractile activity on the symptomatic leg, similar to that seen in patients with unilateral lymphedema, facilitating the diagnosis of a lymphatic etiology of her lymphedema. More important, despite the gross lymphatic anatomy on the subject’s right leg, the functional lymphatic vessel in the ankle may portend responsiveness to standard lymphatic treatments to stimulate the lymphatic pump. NIRFLI definitively and rapidly identified lymphatic anatomic and functional abnormalities and could, in the future, provide evidence of lymphatic dysfunction that may need to be addressed before or simultaneously with hemovascular interventions.

Whereas there is no hemodynamic basis for an outflow stenosis to produce edema in the contralateral limb, it has been postulated that lymphedema praecox or en tarda is perhaps an acquired rather than a congenital disorder. Calnan et al theorized that venous obstruction caused lymphedema praecox and showed abnormal phlebograms
in 11 of 23 lymphedema patients with classic evidence of May-Thurner syndrome and an elevated venous pressure gradient. Although this patient had many clinical features of lymphedema praecox, high-grade venous iliac stenosis in the left leg as a cause of right extremity lymphedema does not seem to explain her edema, especially because stenting did not improve swelling. Because NIRFLI was not conducted before stenting, its effects, if any, on the lymphatic function in the ipsilateral, treated leg are unknown. Likewise, because this study was focused only on characterizing lymphatic contribution, venous imaging was not conducted at the time of NIRFLI for this subject previously treated for May-Thurner syndrome.

CONCLUSIONS

Future work using both venous imaging and NIRFLI to assess changes in venous and lymphatic anatomy and function after stenting could provide a better understanding of the interplay between venous outflow and lymphatic insufficiency and may improve patient management. Our results suggest that NIRFLI provides a nonradioactive, “point-of-care” method to rapidly and economically assess the lymphatic contribution to edema that may be associated with venous disease. With the availability of NIRFLI, we believe clinical research opportunities will emerge to better understand the relative contributions of the lymphatic vasculature in the etiology, presentation, and resolution of venous disease.

The authors thank Erik A. Maus, MD, for his clinical assistance during this study.

REFERENCES

1. O’Donnell TF Jr. Congenital mixed vascular deformities of the lower limb: the relevance of lymphatic abnormalities to their diagnosis and treatment. Ann Surg 1977;185:162-8.

2. Raju S, Owen S Jr, Neglen P. Reversal of abnormal lymphoscintigraphy after placement of venous stents for correction of associated venous obstruction. J Vasc Surg 2001;34:779-84.

3. Foldi M. Foldi’s textbook of lymphology. Philadelphia: Elsevier Health Sciences; 2012.

4. Sevick-Muraca EM. Translation of near-infrared fluorescence imaging technologies: emerging clinical applications. Annu Rev Med 2012;63:217-31.

5. Tan IC, Maus EA, Rasmussen JC, Marshall MV, Adams KE, Fife CE, et al. Assessment of lymphatic contractile function after manual lymphatic drainage using near-infrared fluorescence imaging. Arch Phys Med Rehabil 2011;92:756-64.e1.

6. Zhang J, Zhou SK, Xiang X, Bautista ML, Niccum BA, Dickinson GS, et al. Automated analysis of investigational near-infrared fluorescence lymphatic imaging in humans. Biomed Opt Express 2012;3:1713-23.

7. Swartz MA. The physiology of the lymphatic system. Adv Drug Deliv Rev 2001;50:3-20.

8. Rasmussen JC, Tan IC, Marshall MV, Adams KE, Kwon S, Fife CE, et al. Human lymphatic architecture and dynamic transport imaging using near-infrared fluorescence. Transl Oncol 2010;3:362-72.

9. Burrows PE, Gonzalez-Garay ML, Rasmussen JC, Aldrich MB, Guillod R, Maus EA, et al. Lymphatic abnormalities are associated with RASA1 gene mutations in mouse and man. Proc Natl Acad Sci U S A 2013;110:8621-6.

10. Godkeke PJ, Montgomery RA, Petronis JD, Minken SL, Peeler BA, Williams GM. Lymphoscintigraphy to confirm the clinical diagnosis of lymphedema. J Vasc Surg 1989;10:306-12.

11. Cambria RA, Giovickzi P, Naecens JM, Walner HW. Noninvasive evaluation of the lymphatic system with lymphoscintigraphy: a prospective, semiquantitative analysis in 386 extremities. J Vasc Surg 1993;18:773-82.

12. Mihara M, Hara H, Azaki J, Kikuchi K, Narushima M, Yamamoto T, et al. Indocyanine green (ICG) lymphography is superior to lymphoscintigraphy for diagnostic imaging of early lymphedema of the upper limbs. PLoS One 2012;7:e38182.

13. Calnan JS, Kountz S, Pentecost BL, Shillingford JP, Steiner RE. Venous obstruction in the aetiology of lymphoedema praecox. Br Med J 1964;2:221-6.

Submitted Apr 15, 2015; accepted May 15, 2015.