Published in final edited form as:

J Vasc Surg Venous Lymphat Disord. 2017 July ; 5(4): 533–537. doi:10.1016/j.jvsv.2017.01.011.

Near-Infrared Fluorescence Lymphatic Imaging of Klippel-Trenaunay Syndrome

John C. Rasmussen^{1,*}, Rodrick C. Zvavanjanja^{2,*}, Melissa B. Aldrich¹, Matthew R. Greives³, and Eva M. Seveck-Muraca¹

¹The Brown Foundation Institute of Molecular Medicine, McGovern Medical School at The University of Texas Health Science Center at Houston, Houston, TX

²UTHealth Vascular Anomalies Clinic, Department of Diagnostic and Interventional Imaging, McGovern Medical School at The University of Texas Health Science Center at Houston, Houston, TX

³UTHealth Vascular Anomalies Clinic, Division of Pediatric Plastic Surgery, Department of Pediatric Surgery, McGovern Medical School at The University of Texas Health Science Center at Houston, Houston TX

Abstract

The relationship between lymphatic and venous malformations in Klippel Trenaunay Syndrome is difficult to assess. Herein the authors describe near-infrared fluorescence lymphatic imaging (NIRFLI) to assess the lymphatics of a subject with a large port wine stain and right leg edema. While lymphatic vessels in the medial, affected knee appeared dilated and perhaps tortuous, no definitive abnormal lymphatic pooling or propulsion was observed. The lymphatics in the affected limb were well-defined, though less numerous than in the contralateral limb, and active, contractile function was observed in all vessels. As demonstrated, NIRFLI enables the clinical assessment of lymphatics in lymphovenous malformations.

Keywords

Near-infrared fluorescence imaging; Lymphatics; Klippel-Trenaunay Syndrome

Address correspondence to: John C. Rasmussen, UTHealth, 1825 Pressler Street, Houston, TX 77030; Telephone: 713-500-3393; Fax: 713-500-0319; John.Rasmussen@uth.tmc.edu.

*Denotes equal contribution

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Disclosures: 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. The remaining authors have no financial relationships or conflicts of interest relevant to this article to disclose.

Introduction

While vascular malformations are a hallmark of Klippel Trenaunay syndrome (KTS), patients may present with either venous or lymphatic malformations or a combination of both,[1–3] and an understanding of the contribution of both vasculatures is needed to achieve optimal interventional outcomes. Several established imaging modalities, including venography, magnetic resonance imaging (MRI), and duplex ultrasonography, are commonly employed to assess vascular anatomy and function,[4] however, unidirectional (distal to proximal) flow of lymph, small vessel sizes, and the lack of endogenous contrast inhibit the use of these imaging modalities to assess lymphatic anatomy and function[5]. While lymphoscintigraphy and MRI have been used to assess lymphatic drainage in KTS patients[1,2], they typically do not provide adequate spatial and/or temporal resolution for contractile function assessment. Recent developments in near-infrared fluorescence lymphatic imaging (NIRFLI), which utilizes a non-radioactive, fluorescent contrast agent for rapid image acquisition, provide unique opportunities to assess the lymphatic involvement in KTS in near-real-time. This report is published with consent of the subject.

Case Report

A 32 year old male, diagnosed with KTS, was referred to our Vascular Anomalies Clinic (VAC) for treatment of his complex venous anatomy. The affected right lower extremity had a large port wine stain (Figure 1A–D), soft tissue and bony overgrowth, and history of femoral deep vein thrombosis (DVT). His primary complaint was right lower extremity swelling when standing for long periods. The subject, 1.7 m tall with a BMI of 31.3, had a history of hypertension and hyperlipidemia.

The initial work up, including magnetic resonance imaging (MRI) and ultrasound scans, was performed at an outside hospital. Because of the uncertainty of the lymphatic contribution to his symptoms, the attending VAC physician requested the patient be evaluated for lymphatic malformation or dysfunction. After informed consent, the patient was enrolled into the institutional review board- and Food and Drug Administration-approved study using NIRFLI to assess lymphatic anatomy and contractile function in the broad spectrum of lymphatic disorders and to identify underlying causative genetic mutations (NCT00833599: “Imaging Lymphatic Function in Normal Subjects and in Persons with Lymphatic Disorders,” www.clinicaltrials.gov) and in which 186 subjects have been enrolled to date, with this being the only KTS subject.

NIRFLI was performed following intradermal administration of indocyanine green (ICG) into the dorsum of the feet, medial ankles, lateral and medial calves, and thighs as shown in Figure 1E. Each injection consisted of 25 µg ICG in 0.1 mL saline for a total dose of 300 µg ICG. Immediately following injection, near-infrared (NIR) fluorescent images were acquired using a custom imaging device[6] (Figure 1F) which consisted of a diffused 785 nm laser diode excitation source to excite ICG-laden lymph, producing an 830 nm fluorescent signal. The fluorescent signal was then amplified by a NIR sensitive image intensifier coupled to a charge coupled device (CCD) camera. Image exposure times of 200 ms allowed the visualization of ICG-laden lymph and contractile lymphatic flow in near-real-time.

As shown in Figure 2, the lymphovascularity in the anterior of this subject's legs (Figure 2A–D, Supplemental Videos 1 and 2) appeared linear and well-defined, as seen in healthy subjects previously imaged (Figure 2E), with no evidence of the lymphatic dermal backflow or extravascular fluorescence (Figure 2F) commonly seen in individuals with known lymphatic disorders[7–10]. However, while healthy subjects typically have bilaterally symmetric lymphovascularity (Figure 2E), this subject had about half the number of lymphatics in the affected leg as the unaffected leg. The lymphatics in the medial, affected knee were dilated (Figure 3A, Supplemental Video 3) and appeared more segmented as compared to the medial, unaffected knee (Figure. 3B). After imaging contractile activity in the supine position for approximately 45 minutes, the subject's legs were imaged while standing (Figure 2D).

Active lymphatic propulsion, characterized by the movement of fluorescent boluses of ICG-laden lymph along lymphatic vessels, was observed in both the supine (Supplemental Video 1) and standing (Supplemental Video 2) positions. In an 11 minute observation period, the average time between propulsion events in each functioning lymphatic vessel was 68.8 ± 13.8 sec and 67.6 ± 16.1 sec in the affected and unaffected legs respectively. Similar propulsion rates were observed in both the supine and standing positions.

The reduced number of lymphatic vessels in the affected leg likely results in insufficient lymphatic capacity to handle the fluid burden from the impaired venous output of the combined DVT, varicose veins and valve incompetence identified via standard-of-care venography, MRI and ultrasonography. However, because the lymphatics were well-defined with active lymphatic propulsion, no lymphatic treatment was performed and the subject was ultimately prescribed compression garments as it was determined that the superficial, varicose veins provided collateral drainage around femoral, thrombotic venous occlusions.

Discussion

From observing the flow of ICG-laden lymph through the dilated lymphatics (Supplemental Video 3) it is hypothesized, but not proven, that these medial lymphatics may, like venous varicosities, be tortuous and effectively “corkscrew” towards the inguinal region. This hypothesis may account for the segmented appearance, as the deeper portions of the vessel would appear less bright, owing to light attenuation in tissue. The noted asymmetrical anatomical differences could be due to small variations in the locations of the bilateral injection sites, but they are more likely associated with the unilateral disease as has been noted previously in subjects with lymphedema[8] and in a study of a subject with Parkes Weber syndrome, who displayed unilateral lymphedema (ipsilateral with his port wine stain) of the foot and genitals[11]. In that subject, extensive dermal lymphatic backflow was observed in the affected leg (Figure 2F), along with dilated lymphatics displaying sluggish flow in the unaffected leg.

Maari et al., retrospectively found the type of port wine stain to be indicative of probable lymphatic malformation in KTS patients, with 21 of 22 subjects with “geographic” (sharply demarcated borders) port wine stains and only 1 of 17 subjects with “blotchy” (large expanse without consistently sharp demarcation) port wine stains having probable lymphatic

disease.[1] It is possible, however, that some of Maari's subjects not identified as having lymphatic disease may have had unseen malformations beneath the dermis as Liu et al. observed lymphatic malformations in 31 of 32 subjects with KTS and lymphedema using magnetic resonance lymphangiography[2].

Lymphoscintigraphy and MRI have been used, with varying degrees of success to assess lymphatic drainage in KTS subjects[1,2] and abnormal lymphatics[12,13]. Recent developments in dynamic, contrast enhanced MRI techniques enable the visualization of dynamic movement of a directly administered (into lymphatic vessel or node) contrast agent through the lymphatics and may provide feedback for lymphatic procedures such as lymphatic embolism[14] or sclerotherapy[15]. While these modalities enable the anatomical visualization of deeper (>3–4 cm) lymphatics that may not be seen with NIRFLI, owing to light attenuation in tissue, they typically do not provide adequate spatial and/or temporal resolution for lymphatic contractile function assessment, as demonstrated herein. Additional NIRFLI studies are needed to assess (i) whether other KTS patients demonstrate a similar lymphatic phenotype to that observed in this subject, or if there is a broad spectrum of lymphatic malformation and malfunction; (ii) the contribution of observed lymphatic malformations to the symptoms of KTS; and (iii) whether NIRFLI before and after venous or lymphatic intervention in subjects with lymphovenous malformations may enable the assessment of these procedures' impact on the lymphatics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of Funding: This work was supported in parts by NIH R01 HL092923, U54 CA136404 and the University of Texas Health Science Center Medical School.

References

1. Maari C, Frieden IJ. Klippel-Trénaunay syndrome: the importance of “geographic stains” in identifying lymphatic disease and risk of complications. *J Am Acad Dermatol.* 2004; 51:391–8. [PubMed: 15337982]
2. Liu N-F, Lu Q, Yan Z-X. Lymphatic malformation is a common component of Klippel-Trenaunay syndrome. *J Vasc Surg.* 2010; 52:1557–63. [PubMed: 20800418]
3. Sharma D, Lamba S, Pandita A, Shastri S. Klippel–Trénaunay Syndrome – A Very Rare and Interesting Syndrome. *Clin Med Insights Circ Respir Pulm Med.* 2015; 9:1–4.
4. Bastarrika G, Redondo P, Sierra A, Cano D, Martínez-Cuesta A, López-Gutiérrez JC, et al. New techniques for the evaluation and therapeutic planning of patients with Klippel-Trénaunay syndrome. *J Am Acad Dermatol.* 2007; 56:242–9. [PubMed: 17175065]
5. Sevick-Muraca EM, Kwon S, Rasmussen JC. Emerging lymphatic imaging technologies for mouse and man. *Journal of Clinical Investigation.* 2014; 124:905–14. [PubMed: 24590275]
6. Rasmussen J, Kwon S, Sevick-Muraca E, Cormier J. The Role of Lymphatics in Cancer as Assessed by Near-Infrared Fluorescence Imaging. *Annals of Biomedical Engineering.* 2012; 40:408–21. [PubMed: 22139396]
7. Rasmussen JC, Tan IC, Marshall MV, Fife CE, Sevick-Muraca EM. Lymphatic imaging in humans with near-infrared fluorescence. *Curr Opin Biotechnol.* 2009; 20:74–82. [PubMed: 19233639]

8. Rasmussen JC, Tan IC, Marshall MV, Adams KE, Kwon S, Fife CE, et al. Human lymphatic architecture and dynamic transport imaged using near-infrared fluorescence. *Translational Oncology*. 2010; 3:362–72. [PubMed: 21151475]
9. Rasmussen JC, Fife CE, Sevick-Muraca EM. Near-Infrared Fluorescence Lymphatic Imaging in Lymphangiomatosis. *Lymphat Res Biol*. 2015; 13:195–201. [PubMed: 26287470]
10. Rasmussen JC, Aldrich MB, Tan I-C, Darne C, Zhu B, O'Donnell TF, et al. Lymphatic transport in patients with chronic venous insufficiency and venous leg ulcers following sequential pneumatic compression. *Journal of Vascular Surgery: Venous and Lymphatic Disorders*. 2016; 4:9–17. [PubMed: 26946890]
11. Burrows PE, Gonzalez-Garay ML, Rasmussen JC, Aldrich MB, Guilliod R, Maus EA, et al. Lymphatic abnormalities are associated with RASA1 gene mutations in mouse and man. *Proceedings of the National Academy of Sciences*. 2013; 110:8621–6.
12. Flors L, Leiva-Salinas C, Maged IM, Norton PT, Matsumoto AH, Angle JF, et al. MR Imaging of Soft-Tissue Vascular Malformations: Diagnosis, Classification, and Therapy Follow-up. *RadioGraphics*. 2011; 31:1321–40. [PubMed: 21918047]
13. Witte CL, Witte MH, Unger EC, Williams WH, Bernas MJ, McNeill GC, et al. Advances in Imaging of Lymph Flow Disorders. *RadioGraphics*. 2000; 20:1697–719. [PubMed: 11112825]
14. Itkin, M., McCormack, FX., Dori, Y. Diagnosis and Treatment of Lymphatic Plastic Bronchitis in Adults Using Advanced Lymphatic Imaging and Percutaneous Embolization. *Annals of the American Thoracic Society* [Internet]. 2016. [cited 2016 Oct 28]; Available from: <http://www.atsjournals.org/doi/10.1513/AnnalsATS.201604-292OC>
15. Perkins JA, Manning SC, Tempero RM, Cunningham MJ, Edmonds JL, Hoffer FA, et al. Lymphatic malformations: Review of current treatment. *Otolaryngology – Head and Neck Surgery*. 2010; 142:795–803.e1. [PubMed: 20493348]

Figure 1.

Montages of color images, acquired after injection with indocyanine green, depicting the (A) anterior lower legs, (B) right medial leg, (C) posterior lower legs, and (D) anterior thighs of the subject, as well as the extent of the port wine stain associated with Klippel Trenaunay Syndrome. (E) Diagram illustrating the injection sites which are covered with round bandages and/or black vinyl tape in A–D. (F) Picture of the NIRFLI device with the excitation and fluorescence optics as well as the cameras mounted on the end of the articulating arm while the computer and supporting hardware is stored in the cart.

Figure 2.

Fluorescent images of the lymphatics in the (A) feet and lower shins (see Supplemental Video 2), (B) upper shin and knees, (C) and medial thighs while the subject was supine, and (D) the lower legs while the subject was standing upright (see Supplemental Video 3). The insets in (C) illustrate a better view of the lymphatics draining the injection sites on the thighs, including lymph nodes (arrow) observed in the left thigh. (E) Fluorescent image of the lymphatics in a healthy subject with a similar lymphatic phenotype as this subject. (F) Fluorescent image of the dermal backflow and/or extravascular fluorescence in a subject

with Parkes-Weber syndrome and typically associated with lymphatic disorders. The circles on the inset color images outline the field of view of the fluorescent images. The injection sites are covered with round bandages and/or black vinyl tape.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 3.

Images of the lymphatics in the (A) affected, right (see Supplemental Video 1) and (B) unaffected, left medial knees. The lymphatics in the affected knee is dilated and more segmented in comparison with the unaffected knee. Injection sites are covered with round bandages and/or black vinyl tape.