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Assessment of lymphatic contractile function following manual lymphatic drainage using near-infrared fluorescence imaging

I-Chih Tan, PhD^{1,4}, Erik A. Maus, MD^{2,3}, John C. Rasmussen, PhD^{1,4}, Milton V. Marshall, PhD^{1,4}, Kristen E. Adams, PhD^{1,4}, Caroline E. Fife, MD^{2,3}, Latisha A. Smith, MD^{2,3}, Wenyaw Chan, PhD⁵, and Eva M. Sevick-Muraca, PhD^{1,4,6}

¹ Center for Molecular Imaging, The Brown Foundation Institute of Molecular Medicine at The University of Texas Health Science Center, Houston, TX

² Division of Cardiology and Hyperbaric Medicine, Department of Internal Medicine at The University of Texas Health Science Center, Houston, TX

³ Memorial Hermann Center for Lymphedema Management, Houston, TX

⁴ Formerly, Department of Radiology, Baylor College of Medicine, Houston, TX (where portions of this work were conducted)

⁵ Division of Biostatistics, University of Texas School of Public Health, Houston, TX

Abstract

Objective—To investigate the feasibility of assessing the efficacy of manual lymphatic drainage (MLD), a method for lymphedema (LE) management, using near-infrared (NIR) fluorescence imaging.

Design—Exploratory pilot study.

Setting—Primary care unit.

Intervention—Indocyanine green of 25 µg in 0.1 cc each was injected intradermally in bilateral arms or legs of subjects. Diffused excitation light illuminated the limbs and NIR fluorescence images were collected using custom-built imaging systems. The subjects received MLD therapy, and imaging was performed pre- and post- therapy.

Participants—Ten subjects (age 18 – 68) diagnosed with Grade I or II LE and 12 normal control subjects (age 22 – 59).

Main outcome measures—Apparent lymph velocities and the periods between lymphatic propulsion events were computed from fluorescence images. The data collected pre- and post-MLD were compared and evaluated for differences.

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⁶To whom correspondence should be directed, 1825 Pressler St, SRB 330A, Houston, TX 77030, phone: 713-500-3560; fax: 713-500-0319; eva.sevick@uth.tmc.edu.

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Results—By comparing the pre- MLD lymphatic contractile function against post- MLD lymphatic function, our results show that the average apparent lymph velocity increased in both the symptomatic (+23%) and asymptomatic (+25%) limbs of LE subjects and in the control limbs (+28%) of normal subjects. The average lymphatic propulsion period decreased in the symptomatic (−9%) and asymptomatic (−20%) limbs of LE subjects, as well as in the control limbs (−23%).

Conclusions—We demonstrated that NIR fluorescence imaging could be used to quantify immediate benefits of lymphatic contractile function following MLD.

Keywords

fluorescence imaging; lymphedema; manual lymphatic drainage

While one in 30 persons worldwide suffer from lymphedema (LE) (1), management of this disease has escaped rigorous scientific investigation. LE as a result of cancer staging and treatment has an estimated incidence of up to 50% of all breast cancer patients who undergo axillary lymph node dissection and radiation (2), and up to 64% of those cancer patients undergoing groin or pelvic lymph node dissections (3). A smaller, but equally impacted, population of children and adults with congenital or hereditary lymphovascular defects also suffers from LE. In the U.S., there are several non-pharmacological and non-surgical treatments for LE, but as reported recently by Oremus *et al.* (4) to the US Centers for Medicare and Medicaid Services (CMS), no study has demonstrated sufficient evidence of benefit from any of these treatments. The accepted method to manage LE is through the use of complete decongestive therapy (CDT), which includes manual lymphatic drainage (MLD), compression bandaging, therapeutic exercise, and meticulous skin care. Although its effectiveness remains controversial, MLD is thought to first stimulate lymphatic drainage from receiving lymph node basins, and then presumably stimulate contractile or “pumping” function of the superficial (epifascial) lymphatic system for subsequent drainage (5). Response to MLD is usually measured indirectly through reduction of limb volume using a number of accepted and experimental methods over a period of weeks to months (6). Unfortunately, there is no method available to immediately evaluate efficacy of MLD in a single treatment session. While CDT response rates using limb volumetric measurements were reported to be 67.7% for lower extremity and 59.1% for upper extremity LE subjects over a management period of 12 months (6), there remains no method to (i) predict who will respond to CDT or (ii) measure whether contractile function is indeed enhanced by MLD. Unfortunately, clinical demonstration of lack of MLD efficacy requires disease progression, because there are no or few scientific measures of efficacy possible within a single treatment session.

Lymphoscintigraphy is the only accepted imaging approach for diagnosis of lymphatic transport dysfunction through quantifying the transit time of radionuclide transport from a distal injection site to the draining lymph node basins, its clearance from the injection site, or its accumulation in draining lymph nodes (7). Magnetic resonance imaging (MRI) (8) and computed tomography (CT) (9) are also used when lymphoscintigraphy is not available. Insufficient spatial and temporal resolutions of these imaging modalities, as well as lack of contrast agent clearance, coupled with poor temporal resolution, do not allow pre- and post-MLD imaging in a single therapy session to provide efficient evaluation of LE treatment (10).

Recently, we have developed an investigational technique of near-infrared (NIR) fluorescence imaging that has tenths-of-second temporal resolution to visualize the active contractile function and architecture of human lymphatics (11). Because a fluorophore can be repeatedly excited to provide significant photon count rates, *in vivo* human imaging can

be accomplished using microdose administration of fluorescent contrast agent (12). Marshall *et al.* have provided an extensive review of several studies using NIR optical imaging devices and indocyanine green (ICG) to map the lymphatics in humans (11, 13, 14). Previously, we have shown that the architecture and contractile function of lymphatics can be directly visualized to show differences in lymph transport between normal limbs of control subjects, asymptomatic limbs of LE subjects, and symptomatic limbs of LE subjects (15, 16). The differences in lymphatic contractile function were quantified (i) by the apparent propulsive lymph velocities and (ii) by the period or time of arrival between successive propulsive events. This was the first study of NIR fluorescence imaging used to assess lymphatic contractile function in human. No other imaging modality exists to provide quantifiable metrics of lymphatic (dys)function.

A feasibility study was designed to test the hypothesis that NIR fluorescence imaging could quantitatively evaluate the responses of lymphatic contractile function pre- and post- MLD in normal control subjects and in persons clinically diagnosed with extremity LE. We sought to determine whether NIR fluorescence imaging could be used to evaluate effectiveness of MLD immediately after a single therapy session.

Methods

Study Design

The protocol used for this exploratory pilot study was approved under a combinational exploratory investigational new drug (eIND) application for the off-label use of indocyanine green (ICG) as a NIR fluorescent contrast agent. The HIPPA-compliant studies were approved by the Institutional Review Board (IRB) at XXXXX, where the trials were conducted, and at the XXXXX, where the imaging data was analyzed. This pilot study was limited to determine the feasibility of using NIR fluorescence imaging for assessing response to MLD. Twelve normal volunteers and 10 subjects clinically diagnosed with Grade I or II unilateral LE participated and provided informed consent. All subjects received MLD therapy. The detail demographics and disease etiology of these subjects are available in Table I.

Near-Infrared Fluorescence Imaging

The imaging contrast agent, ICG, was reconstituted in water and diluted in saline to a concentration of 0.25 mg/ml. Each subject received intradermal injections of 0.1 ml each using 29-gauge needles for a maximum total dose of 400 µg ICG. Injection sites were symmetrical on contralateral limbs. The maximum number of injections was 6 in each arm and 8 in each leg. Generally, 2 injections were made on the dorsum of the hand, 2 on the medial forearm, and 2 on the lateral forearm when arms were imaged. When imaging legs, 2 injections were made in the dorsum of the foot, 2 on the medial ankle, 1 on the heel, 2 on the calf, and 1 on the thigh. The subjects were given the options to receive topical anesthetic with lidocaine 2.5% and prilocaine 2.5% cream applied directly to the injection site to lessen the injection sensation as prior work showed no difference in measurements with and without topical anesthetic (15). All subjects were asked whether they wished to decline further injections in the event they found the first injections uncomfortable. Immediately after injections, the location and movement of ICG in the lymphatics were imaged simultaneously in both limbs using two custom-built fluorescence imaging systems. Details of the imaging systems can be found elsewhere (15), but in brief, each system consisted of an excitation light source, holographic and band-pass filters, a NIR sensitive image intensifier, and a customized charge coupled device (CCD) camera. Sequential fluorescence images were acquired using a 200 msec camera integration time permitting near real time imaging of the lymphatics. Subjects remained supine on a bed during imaging. After ICG

administration, the subjects were first imaged for 30 to 60 minutes. Then, MLD was performed for 23 – 28 minutes with concurrent imaging. Imaging continued 30 to 60 minutes after the finish of MLD. No adverse events were associated with the imaging agent or device in this study. Apparent lymph velocities and propulsion periods were calculated from the imaging data (15).

Manual Lymphatic Drainage (MLD)

MLD was performed on bare skin by a certified LE therapist; neither oils nor lotions were used during MLD therapy. MLD consisted of gentle massage to the cervical lymph nodes for 3 minutes, followed by massage to the axillary and inguinal nodes for 5 minutes in the preparation period. In subjects with arm LE, the areas treated with massage were the neck, followed by the axillary region on the contralateral (asymptomatic) arm, and the ipsilateral (symptomatic side) inguinal region. For leg LE subjects, the procedure consisted of massage at the neck, followed by treatment of the contralateral inguinal nodes and ipsilateral axillary lymph nodes. For control subjects, following massage to the neck for 3 minutes, massage was performed at the bilateral axillary regions for 5 minutes if the arms were imaged, or at the bilateral inguinal regions if the legs were imaged.

After this preparation period, the limbs being imaged were massaged with centripetal light strokes starting at the proximal aspect of the limb, following with more distal segments. For the arms, the MLD protocol for both control and LE subjects was conducted in the following order: (i) 5 minutes on the upper arm, (ii) 5 minutes on the forearm, and (iii) 5 minutes on the hand. Similarly for the legs, the order was (i) 5 minutes on the thigh, (ii) 5 minutes on the leg, and (iii) 5 minutes on the foot. Live fluorescence images of lymphatic architecture were available to therapists during MLD to provide guidance for massage. The images collected before MLD were classified as “pre-MLD”, and the data after as “post-MLD” results. Apparent lymph velocity and periods between successive propulsions of lymph were computed from the movement of fluorescent packets in images as in previous study (15).

Statistical Analysis

Statistical analysis of the apparent velocities and propulsion periods computed from the images was performed using MATLAB and SAS. Each propulsion of ICG-laden lymph was assumed to be an independent event for a given subject, and the distributions of apparent velocities and propulsion periods were assumed to be log-normal. Although a larger clinical trial will enable determination of whether the latter assumption is correct, this assumption alleviated the data skewness and made the observations more symmetric. The Kolmogorov-Smirnov test indicates that at least half of the subjects had their apparent velocities and propulsion periods follow the normal distribution after log-transformation, and the remainder did not have a small enough p-value for rejection of normality. Analysis of variance (ANOVA) of the logarithm-transformed data were performed to determine the relationship of lymphatic functions between pre- and post- MLD for different diagnoses (control, symptomatic, and asymptomatic) and limbs (arm and leg). For data from each individual subject, paired t-tests were performed to investigate the effect of MLD on each side (“right” or “left” for control subjects, or “symptomatic” or “asymptomatic” for LE subjects). For study-wide analysis, the data were pooled by groups of interest (i.e. all asymptomatic arm data versus all symptomatic arm data) to investigate differences between groups. Due to the nature of correlated observations of apparent velocities and propulsion periods within subjects, analyses using linear mixed model for repeated measurements were performed. For all analyses, the tested factor was determined to be significant when the p-value was less than 0.05.

Results

Since this is an exploratory pilot study, the sample size is limited and not powered for statistical testing. Nonetheless, even though comprehensive comparisons were not intended, our data provides sufficient numbers for partial evaluation. Specifically, we found statistically significant differences pre- and post- MLD in normal and LE subjects, as well as trends of improved lymph transport post-MLD that suggest (i) the benefit of MLD and (ii) the use of investigational NIR fluorescence imaging to potentially provide direct evidence of benefit from MLD in LE subjects within a single therapy session. The statistics of lymph function in arms and legs for pooled data are summarized in Table II. In the following, we present results detailing the effect of MLD in both the arms and legs of normal and LE subjects.

Effect of manual lymphatic drainage in arms

Images of lymphatics show striking differences in lymphatic vasculatures between control limbs and symptomatic and asymptomatic LE limbs which is similar to what was observed in our previous study (15). The lymphatic structure in the limbs of normal subjects generally consists of well-defined channels that actively propel and drain lymph into the regional nodal basins. In contrast, the lymphatic structure of symptomatic limbs of LE subjects typically displays capillary networks, tortuous vessels, or extravascular lymphatic fluid leakage.

Figure 1A shows an example fluorescence image obtained from the control arm of subject NA12 when a gentle massage stroke of MLD was performed on the right forearm. Figure 1B shows the fluorescent image obtained 9.3 s after the massage stroke. All images presented herein are in pseudo-color. A wave of fluorescent lymph “packets” (indicated by arrows) was observed in multiple vessels moving toward axillary lymph nodes. Abbreviated and example compilation of these images are shown in supplemental videos 1 and 2. Supplemental video 3 shows a similar image sequence recorded during MLD on the symptomatic arm of LE subject LA03. All videos are played approximately 3 times faster than real time. From the full length image sequences exemplified above, the values of apparent lymph velocity and propulsion periods were determined..

The averages of the pre- and post- MLD apparent lymph velocities and propulsion periods obtained from the symptomatic and asymptomatic arms of 5 LE subjects and the normal (both right and left) arms of 6 control subjects are shown in Figure 2. Significant differences ($p < 0.05$) between the velocities or periods measured pre- and post- MLD within each subject are marked with asterisks. In the normal control subjects, 5 out of 6 subjects experienced trends of increased apparent lymph velocities after MLD, with 3 out of 5 subjects experiencing statistically significant differences in one or both arms. Of the 6 normal subjects, 4 experienced trends of reduced propulsive periods (or increased propulsive frequencies) after MLD, indicating improved lymphatic transport. Of those 4 normal subjects, 2 showed statistically significant improvement in propulsive periods. It is noteworthy that, as expected, there is no statistically significant reduction in lymphatic transport (i.e. decrease in apparent lymph velocity *or* increase in propulsive period) in normal subjects. However, one subject (NA11) tended to have changes that indicate reduction of lymphatic transport following MLD (i.e., reduction of apparent velocity and increase in propulsive period), suggesting the variability of MLD effectiveness reported in the literature. These results show that future clinical trials need to be designed to assess whether individual variability in the immediate response to MLD in the normal population and within a single session, mimics the variability similarly assessed in well defined LE populations.

In the LE group, the average apparent lymph velocity in all asymptomatic arms increased after MLD, with one showing a statistically significant increase. In the 4 of 5 symptomatic arms that demonstrated propulsive lymphatic function, two showed increased average apparent lymph velocity after MLD, although the other 2 demonstrated reduction in apparent velocity. The asymptomatic arm of subject LA04 and the symptomatic arm of subject LA05 showed statistically significant increases in propulsion period, while the symptomatic arm of subject LA04 showed statistically significant decreases in propulsive period following MLD, indicative of improvement of lymphatic transport. Two of the 5 LE subjects (LA04 and LA06) experienced improved function, as indicated by the increase in apparent velocity and decrease in propulsive period in symptomatic arms following MLD. It is noteworthy that subject LA06 was clinically responsive to intensive MLD and bandaging. In this subject, the volume of the afflicted arm decreased by 15% after every first week of three 6-week LE treatment sessions.

No propulsion was found in the symptomatic arms of either subjects LA01 or LS03, implying ineffectiveness of MLD. Dermal backflow was observed in both of these subjects' hands, providing possible speculation for the clinically demonstrated failure of MLD effectiveness in the management of their disease. Indeed, subject LA01 was clinically unresponsive to intensive MLD and bandaging conducted over a period of 6 weeks.

The statistical results of the pooled data grouped into symptomatic, asymptomatic, and control arms are shown in Figure 3. The average apparent lymph velocities and propulsion periods are plotted for each group, with the percentages of changes from pre- to post- MLD indicated. The apparent lymph velocities show trends of increase for all groups after MLD, and the increases were statistically significant in asymptomatic and control arms using the linear mixed model (see Table II). The periods show trends of decrease for all groups, and the decrease for the control arm group was statistically significant. Overall, our results show significant increase in apparent velocity and decrease in period after MLD on arms.

Effect of manual lymphatic drainage in legs

Figure 1C shows a fluorescence image obtained from the symptomatic leg of subject L13 when MLD was performed on the foot. Figure 1D shows the image obtained 2.8 s afterward. Lymph in a vessel was observed being pushed toward the ankle due to the massage strokes. The compilation of these images is shown in supplemental video 4. Also, the enhanced lymphatic contractile function of a symptomatic leg during MLD is demonstrated in supplemental video 5.

The averages of the pre- and post- MLD apparent lymph velocities and propulsion periods obtained from the symptomatic and asymptomatic legs of 5 LE subjects and the control legs of 6 normal subjects are shown in Figure 4. In 10 out of 12 control legs, there was an increase in average apparent velocity after MLD, with 5 exhibiting statistically significant increases. The periods showed statistically significant decrease after MLD in 4 control legs, indicating an improvement in lymphatic function following MLD.

In this feasibility study, none of the symptomatic legs showed a statistically significant change in apparent lymph velocity after MLD, and one asymptomatic leg (subject LL02) showed a significant decrease in apparent lymph velocity. None of the legs of LE subjects showed significant changes in propulsion period. However, 3 LE subjects (LL02, LL06, and LL07) experienced trends of improved function via either increase in average apparent velocity and/or decrease in average propulsion period in symptomatic legs. It is noteworthy that subject LL02 was clinically responsive to intensive MLD and bandaging. The afflicted limb of subject LL02 showed more than a 12% volume decrease after the first week of a 4-week LE treatment session. LE subject LL01 was not clinically responsive to MLD, and no

lymph propulsion was found in the symptomatic leg, but a single lymph vessel, which was not seen prior to MLD, was recruited for transiting ICG to the trunk.

The statistical results of the pooled data grouped into symptomatic, asymptomatic, and control legs are shown in Figure 5. Average apparent lymph velocities and propulsion periods are plotted for each group, and the percentages of changes from pre- to post- MLD are indicated. The apparent lymph velocities show trends of increase for all groups after MLD, and the increases were statistically significant in control legs (see Table II). The propulsion periods show trends of decrease in asymptomatic and control legs, but are not statistically significant. Over all, the results show statistically significant increase in apparent velocity and trend of decrease in period after MLD on legs.

For the statistical analysis results of the pooled data from all 22 subjects (44 limbs) in the study, ANOVA indicates that MLD improved the lymphatic contractile function, as reported by increases in apparent velocities in all limb diagnoses (symptomatic +23% $p=0.24$, asymptomatic +25% $p=0.007$, and control +28% $p<0.001$), and also by reduction in the propulsion period (symptomatic -8.6% $p=0.37$, asymptomatic -20% $p=0.7$, and control -23% $p=0.007$).

Discussion

MLD is hypothesized to stimulate lymphatic contractile function and promote the clearance of lymph fluid from the affected area (17). Yet some LE patients do not benefit from MLD, and there is a need to provide an imaging tool to predict a patient's benefit from MLD. Medicare reimbursement policies require clinical demonstration of MLD ineffectiveness before certain classes of pneumatic compression devices (PCD) can be prescribed to replace or augment MLD. Such prognostic tools could improve patient compliance, which is most cited as the leading cause of failure of MLD (18), and more efficiently enable the prescription of replacement or augmented therapies.

NIR fluorescence imaging allowed us not only to visualize the lymphatic structures, but also to assess contractile function by measuring apparent lymph velocity and period of propulsion. By evaluating the differences of lymphatic architecture in different diagnosed (symptomatic, asymptomatic, and normal control) limbs and lymphatic contractile function in response to MLD, we sought to assess the effect of MLD on the lymphatics in this Phase 0, exploratory pilot study to establish feasibility of NIR fluorescence lymphatic imaging.

Depending upon the severity of disease, the numbers of functional lymphatic vessels can vary within different regions of the afflicted limb. By directing lymphatic drainage towards existing functional lymphatic structures, improved responses to MLD could result (19). The importance of existing lymphatic vasculature in MLD treatment of early disease is supported by the studies of McNeely *et al.* (20), who found that patients with mild breast-cancer-related LE have significantly larger relative volume reduction following treatment than those with severe LE. Functioning lymphatic vessels that transport lymph towards major lymph node basins may be necessary for LE patients to optimally benefit from MLD. Upon using NIR fluorescence imaging to find functioning lymphatics, manually guide drainage towards them, and treat patients earlier, more effective MLD could potentially result. We have shown that in 10 LE subjects, 2 (LA04 and LA06) out of 5 symptomatic arms and 3 (LL02, LL06 and LL07) out of 5 symptomatic legs showed improved lymphatic function in terms of increased apparent velocity and/or reduced propulsion period following MLD, suggesting MLD is a viable treatment for LE in these subjects. NIR fluorescence imaging could provide guidance for personalized care.

However, the stimulation of contractile function in the symptomatic limbs was quantitatively not as large as that obtained in control limbs, possibly due to the lack of organized lymphatic networks associated with the advancing stage of the LE subjects, which was not controlled for in this pilot study (21). Capillary networks and tortuous vessels in LE may cause high resistance lymph drainage pathways that impede lymph flow [18], even under MLD stimulation. These high resistance drainage pathways may characterize progressive LE and could prevent interstitial fluid from entering collecting vessels, resulting in the extravascular ICG-laden lymphatic fluid that was commonly seen in symptomatic limbs in our studies. The response of lymphatic parameters of velocity and period to MLD in asymptomatic limbs was also reduced in comparison to the control limbs. This result is supported by the abnormal lymphatic architecture observed in asymptomatic limbs and the recent evidence of a systemic or genetic predisposition for acquired lymphedema after surgery or trauma (22). It may also be noteworthy that only the control limb group showed significant decreases in propulsion period after MLD. If partial or complete loss of lymphatic contractile function is associated with progressive lymphedema (21), then stimulation with MLD may be expected to cause a diminished impact on the frequency of lymph propulsion in LE subjects as compared to normal control subjects.

These studies could suggest the feasibility of using NIR fluorescence imaging to evaluate effectiveness of MLD in a single session. The results contained herein may be used to properly power a clinical study to assess both the immediate and long-term benefit of MLD for individuals suffering from LE. NIR lymphatic fluorescence imaging may provide a means to assess best management practices for LE, whether manual physical therapies such as MLD, PCD (16), microsurgery, or new pharmacological targets, to reverse disease progression.

Study limitations

There are study limitations associated with this Phase 0 exploratory and pilot study.

The limited numbers of subjects intentionally does not allow for design of a study, but does provide information to sufficiently power future clinical trials for reaching statistical conclusions that may translate into clinical practice. One could speculate from the results of this study that legs respond better to MLD than arms, and that some subjects benefit more than others. This idea must be borne out in a properly powered longitudinal study of MLD effectiveness. The mechanisms governing therapeutic response could involve the etiology, degree of damage in lymphatics, and the stage of the disease, all of which are not controlled in this study. The diversity in disease etiology and staging in subjects, as well as the protocol design that could not benefit from any prior knowledge, limited this feasibility study. As a consequence, it might be expected that the different responses to MLD for subjects with various disease stage and etiology could not be studied. Due to the limited number of study subjects, the lack of significance of metrics probably results from insufficient power. In addition, our assumption of normality of the apparent velocities and propulsion periods for ANOVA and paired t-test require further investigation for properly powered studies. Nonetheless, the results show the effectiveness of MLD after a single session and suggest a clinical trial designed to statistically address the use of NIR fluorescence imaging metrics for individualized prediction of LE treatment response. Future protocols to acquire sufficient data for evaluating differences in pre- and post- MLD within individual patients could lead to personalized care of lymphedema patients.

Another inherent study limitation remains the lack of independent gold-standard techniques to validate the (i) lymphatic contractile activity as well as (ii) depth of the functional lymphatics imaged by NIR fluorescence imaging. As described above and elsewhere (12), no other imaging technique exists with the temporal discrimination to visualize contractile

activity, which is proposed to be stimulated by MLD. Even so, the approved imaging of lymphatics using lymphoscintigraphy is not amenable for pre- and post- treatment imaging within a single therapy session as is NIR fluorescence imaging. Finally, another limitation inherent to this pilot study may be the insufficient information and mismatched resolution to co-register planar NIR fluorescence imaging with the only other approved lymph imaging approach of planar lymphoscintigraphy to assess depth of lymphatic vessels imaged. While we are developing unique dual labeled imaging agents for positron emission tomography (PET) and near-infrared fluorescence tomography (23, 24) to validate the depth of NIR signal origin, estimates for NIR fluorescence have ranged as high as 4 – 5 centimeters (25). The depth to which lymphatics can be imaged with NIR fluorescence imaging in these studies remains unclear. Nonetheless, we speculate that lymphatics within a few centimeters of the tissue surface were visualized and that our results may not include the response of deep lymphatic beyond the epifascial lymphatics that MLD treatment focused upon improving.

Conclusions

In conclusion, we have demonstrated that NIR fluorescence imaging could be used to quantitatively assess the lymphatic propulsion following MLD due to (i) the temporal resolution that enables visualization of contractile function, (ii) the ability to track lymphatic parameters pre- and post- therapy within the same session, and (iii) the unique quantification of apparent lymph velocity and propulsion period owing to contractile lymphatic transport function. The results of this exploratory pilot study not only show that contractile lymphatic function was improved after a single session of MLD in some subjects, but also demonstrate the feasibility for using NIR fluorescence imaging to monitor response to treatment. Further studies are needed to confirm the prognostic clinical capabilities of NIR fluorescence lymph imaging for assessing best management practices for LE.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The roles of the authors were as follows: ICT, JCR, and KEA image acquisition; EAM manual lymphatic drainage treatment; ICT and JCR image analysis; WC statistical analysis; MVM regulatory aspects and study monitor/coordinator; EAM, CEF and LAS clinical diagnosis and review/interpretation of imaging, guidance of therapy; ICT statistical analysis; EMS, MVM, and CEF conceived and designed study; EMS and CEF oversaw entire study; ICT and EMS wrote paper.

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Figure 1.

NIR fluorescence images during MLD. (A) and (B), sequential images of a wave of fluorescent packets (indicated by arrows) in multiple vessels moving toward axillary lymph nodes resulting from MLD in a control arm (see supplemental video 1), and (C) and (D), sequential images of lymph in a vessel (indicated by arrow) being pushed toward ankle during MLD in a symptomatic leg (see supplemental video 4).

Figure 2.

Lymphatic contractile function on arms. Average apparent lymph velocities and periods of lymphatic propulsion on different arms in lymphedema (symptomatic and asymptomatic) and control (left and right) subjects pre- and post- MLD. (*: $p < 0.05$)

Figure 3. Statistical results of lymphatic contractile function on arms. Average apparent lymph velocities and periods of lymphatic propulsion in different groups (symptomatic, asymptomatic, and control) pre- and post- MLD. The changes in percentage from pre- to post- MLD are labeled. (*: $p < 0.05$)

Figure 4.

Lymphatic contractile function on legs. Average apparent lymph velocities and periods of lymphatic propulsion on different legs in lymphedema (symptomatic and asymptomatic) and control (left and right) subjects pre- and post- MLD. (*: $p < 0.05$)

Figure 5. Statistical results of lymphatic contractile function on legs. Average apparent lymph velocities and periods of lymphatic propulsion in different groups (symptomatic, asymptomatic, and control) pre- and post- MLD. The changes in percentage from pre- to post- MLD are labeled. (*: $p < 0.05$)

Table 1

Subject demographics, ICG dose, location of imaging, and diagnosis.

Subject ID	Age	Gender	Ethnicity *	Dose (µg ICG)	Limb Imaged	Diagnosis
LA01	46	F	C	400	Arm	LE: onset after mastectomy
LA03	62	F	C	300	Arm	LE: onset after mastectomy (right), had 2nd mastectomy (left) 9 years after 1st.
LA04	48	F	C	312.5	Arm	LE: onset after melanoma surgery
LA05	56	F	H	325	Arm	LE: onset after mastectomy
LA06	68	F	C	300	Arm	LE: onset after mastectomy
LL01	18	F	C	400	Leg	LE: onset at age 18 with unknown etiology (no trauma or surgery)
LL02	66	F	C	400	Leg	LE: onset after vulvar cancer surgery.
LL05	23	F	AA	400	Leg	LE: onset occurred after bug bite
LL06	48	F	AA	350	Leg	LE: unknown etiology, possibly due to pressure from large, untreated fibroid cyst in uterine system.
LL07	40	F	AA	400	Leg	LE: onset after spider bite on toe, also had fibroid cysts in groin
NA07	36	F	C	200	Arm	Control normal
NA08	48	F	C	200	Arm	Control normal
NA09	35	F	AA	200	Arm	Control normal
NA10	41	F	H	300	Arm	Control normal
NA11	35	F	C	300	Arm	Control normal
NA12	37	M	A	300	Arm	Control normal
NL06	35	F	C	400	Leg	Control normal
NL08	40	F	A	400	Leg	Control normal
NL09	25	F	C	400	Leg	Control normal
NL10	34	F	C	400	Leg	Control normal
NL11	22	M	C	400	Leg	Control normal
NL12	59	F	C	400	Leg	Control normal

* A=Asian, AA=African American, C=Caucasian, and H=Hispanic

Table II

Pooled data statistics of lymph function.

Group (number of limb)	Pre- MLD				Post- MLD				Post- vs. Pre- MLD			
	# of v	Mean and standard deviation of velocity (cm/s)	# of p	Mean and standard deviation of period (s)	# of v	Mean and standard deviation of velocity (cm/s)	# of p	Mean and standard deviation of period (s)	Change of mean velocity	p-value of velocity *	Change of mean period	p-value of period *
Symp Arm (5)	55	1.04±0.81	38	29.9±18.0	85	1.19±0.69	54	29.6±24.8	+13.6%	0.472	-1.1%	0.932
Asym Arm (5)	129	0.68±0.29	60	42.1±31.4	293	0.87±0.34	170	33.8±24.7	+29.1%	0.002	-19.8%	0.764
Control Arm (12)	798	0.70±0.32	481	56.6±40.5	802	0.80±0.40	440	41.6±32.3	+15.4%	<0.001	-26.6%	0.007
Symp Leg (5)	28	0.62±0.32	13	73.6±60.9	40	0.95±0.66	24	55.4±45.4	+52.7%	0.211	-24.7%	0.311
Asym Leg (5)	47	0.71±0.35	17	70.8±65.5	56	0.76±0.59	28	70.6±53.5	+7.4%	0.730	-0.2%	0.674
Control Leg (12)	297	0.94±0.80	123	53.9±37.4	469	1.27±1.11	281	46.3±40.0	+35.4%	<0.001	-14.1%	0.376
Limb (adjusted by diagnosis)												
Arm	982	0.71±0.37	579	53.4±39.3	1180	0.85±0.42	664	38.6±30.2	+18.9%	<0.001	-27.7%	0.005
Leg	372	0.89±0.74	153	57.5±44.0	565	1.20±1.05	333	49.0±42.1	+35.2%	<0.001	-14.7%	0.490
Diagnosis (adjusted by limb)												
Symp (10)	83	0.90±0.71	51	41.0±38.7	125	1.11±0.68	78	37.5±34.4	+23.2%	0.244	-8.6%	0.373
Asym (10)	176	0.68±0.31	77	48.4±42.9	349	0.85±0.39	198	39.0±32.9	+24.9%	0.007	-19.5%	0.679
Control (24)	1095	0.76±0.51	604	56.1±39.9	1271	0.98±0.78	721	43.4±35.5	+28.0%	<0.001	-22.6%	0.007

of v=Number of velocity measurements, and # of p= Number of period measurements Symp=Symptomatic, and Asym=Asymptomatic

* Evaluated using linear mixed model for repeated measurements