A Novel Approach to Quantifying Lymphatic Contractility during Indocyanine Green Lymphangiography

Melisa D. Granoff, B.A.
Anna Rose Johnson, M.P.H.
Bernard T. Lee, M.D., M.B.A., M.P.H.
Timothy P. Padera, Ph.D.
Echoe M. Bouta, Ph.D.
Dhruv Singhal, M.D.

Boston, Mass.

4 DIAGNOSTIC

Summary: Lymphedema arises from impaired lymphatic function. Quantification of lymphatic contractility has previously been shown using a custom-built near-infrared imaging system. However, to broaden the clinical use of functional lymphatic measurements, these measurements need to be performed using a standard-of-care, clinically available camera. The authors propose an objective, algorithmic, and clinically accessible approach to quantify lymphatic contractility using a 3-minute indocyanine green lymphangiograph recorded with a commercially available near-infrared camera. A retrospective review of the authors' indocyanine green lymphangiography video repository maintained in a Research Electronic Data Capture database was performed. All patients with a newly diagnosed unilateral breast cancer undergoing preoperative indocyanine green lymphangiography were included in the analysis. Patient medical records were then analyzed for patient demographics, and videos were analyzed for contractility. Seventeen consecutive patients with unilateral breast cancers underwent video processing to quantify lymphatic contractility of the ipsilateral extremity in contractions per minute. All patients were women, with an average age of 60.5 years (range, 38 to 84 years). The average lymphatic contractility rate was 1.13 contractions per minute (range, 0.67 to 2.5 contractions per minute). Using a clinically accessible standard-of-care device for indocyanine green lymphangiography, the authors were able to determine lymphatic contractility rates of a normal extremity. The authors' finding falls within the range of previously published data quantifying lymphatic contractility using a research device, suggesting that the authors' technique provides a clinically accessible, time-effective means of assessing lymphatic contractility. Potential future applications include both lymphedema surveillance and evaluation of nonsurgical and surgical interventions. (*Plast. Reconstr. Surg.* 144: 1197, 2019.)
CLINICAL QUESTION/LEVEL OF EVIDENCE: Diagnostic, IV.

Indocyanine green lymphangiography has become a routine component of lymphedema evaluation.[1–3] Indocyanine green lymphangiography facilitates the visualization of lymphatic channels using near-infrared imaging to a depth of 700 to 1000 nm.[3] A significant limitation of indocyanine green lymphangiography is that the interpretation of images, and subsequent correlation with disease stage, is subjective.

Previous attempts have been made to standardize and quantify indocyanine green lymphangiograms, including an evaluation of transit times.[4–6] Furthermore, another method went beyond anatomical imaging and quantified lymphatic contractile function using near-infrared imaging.[7]

From the Department of Surgery, Division of Plastic and Reconstructive Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School; and the Department of Radiation Oncology, Edwin L. Steele Laboratory, Massachusetts General Hospital.
Received for publication November 17, 2018; accepted March 19, 2019.
Presented at the Lymphedema Symposium hosted by the Beth Israel Deaconess Medical Center/Harvard Medical School, in Boston, Massachusetts, November 2 through 3, 2018.

DOI: 10.1097/PRS.0000000000006176

Disclosure: *The authors have no financial interest to declare in relation to the content of this article. The authors did not receive any funding for this study.*

Related digital media are available in the full-text version of the article on www.PRSJournal.com.

However, this approach, although objective and widely applicable within the laboratory setting, has limited clinical utility. The technique required 30 to 120 minutes of imaging, 24- to 48-hour follow-up, and a custom imager not commercially available.[2,7]

Today, the Hamamatsu near-infrared photodynamic eye device (Hamamatsu Corp., Hamamatsu, Japan) has become indispensable in lymphatic surgery. The first description of indocyanine green lymphangiography for the evaluation of lymphatics using the photodynamic eye occurred in 2007.[3] We propose that the photodynamic eye can be used for rapid evaluation of lymphatic contractility. As the field of lymphatics strives to bring objectivity into the characterization and evaluation of the lymphatic system, a clinically practical method of assessing lymphatic contractility could have significant implications in both surveillance of lymphatic function and evaluation following lymphatic procedures. Previous studies have found that symptomatic lymphedema patients have decreased metrics of lymphatic contractile function (velocity and period), further reinforcing this as a potential avenue for objective clinical evaluation.[7]

Using the photodynamic eye to capture 3-minute indocyanine green videos of patients, we propose an algorithmic approach to quantify lymphatic contractility. We hypothesize that the photodynamic eye can rapidly acquire data in the clinical setting to determine lymphatic contractility.

PATIENTS AND METHODS

A retrospective review of our indocyanine green lymphangiography video repository maintained in a Research Electronic Data Capture database was performed.[8] Beth Israel Deaconess Medical Center Institutional Review Board approval was obtained (Protocol No. 2018P000648). All patients with unilateral breast cancer undergoing preoperative indocyanine green lymphangiography[9] were included for analysis. (**See Table, Supplemental Digital Content 1**, which shows patient characteristics and measurements, ***http://links.lww.com/PRS/D779***.) Corresponding medical records were retrieved, and patient demographics and cancer and treatment characteristics were analyzed. Lymphangiography videos were analyzed for contractility.

Indocyanine Green Lymphangiography Technique

Under sterile conditions, 0.1 cc of a 0.625-mg/cc solution of indocyanine green with albumin was injected intradermally into the first and fourth web spaces of the hand, volar forearm, and lateral upper arm of the ipsilateral extremity. The near-infrared camera was fixated on a Mitaka PDE Flex Arm (Mitaka Kohki Co Ltd., Tokyo, Japan) 10 cm above the dorsum of the patient's hand, such that both injection sites were visible. (**See Figure, Supplemental Digital Content 2**, which demonstrates the surgical setup, ***http://links.lww.com/PRS/D780***.) Three-minute indocyanine green lymphangiograms of the dorsum of the hand were recorded immediately after injection.

Video Analysis

Lymphangiography videos were imported into VLC (VideoLAN, Paris, France) and JPEG images of each frame were extracted using the scene filter. Extracted images were uploaded into ImageJ (National Institutes of Health, Bethesda, Md.) and stabilized using the StackReg plug-in (National Institutes of Health, Bethesda, Md.). A minimum of two regions of interest were manually selected on the brightest visible lymphatic channel in closest proximity to the injection site. Average pixel signal intensity was recorded and plotted in Microsoft Excel (Microsoft Corp., Redmond, Wash.) (Fig. 1), then uploaded into MATLAB (MathWorks, Natick, Mass.), and the Peakfinder script (Copyright © 2016, Nathanael C. Yoder) was used to identify signal intensity peaks. [**See Video (online)**, which shows generation of the contractility curve.] Peakfinder source code is available at https://www.mathworks.com/matlabcentral/fileexchange/25500-peakfinder-x0-sel-thresh-extrema-includeendpoints-interpolate. The number of peaks was divided by the length of the video (in minutes) to yield the contractility rate in contractions per minute. The second region of interest on the same channel was used to assess reproducibility of results and eliminate noise introduced from exposure and video variation (Figs. 2 and 3).

RESULTS

Seventeen patients with unilateral breast cancer underwent preoperative indocyanine green lymphangiography of the dorsum of the hand. All patients were women and had a mean age of 60.5 ± 14.2 years. The average contractility rate was 1.13 contractions per minute (range, 0.67 to 2.5 contractions per minute). In all cases, a minimum of two regions of interest from the same channel confirmed the contractility rate.

DISCUSSION

Using clinical imaging equipment, we detected and quantified lymphatic contractility

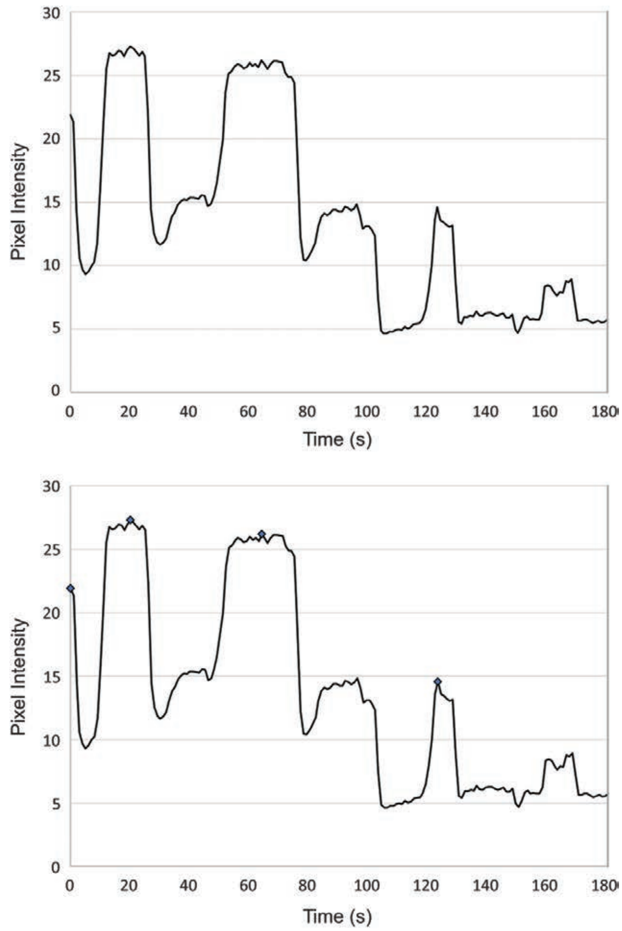

Fig. 1. Contractility curve with automatic peak detection. (*Above*) Contractility curve shows the pixel signal intensity from the region of interest selected in ImageJ. (*Below*) Automatically identified peaks in the contractility curve correspond to lymphatic contractions, and are identified with a blue marker.

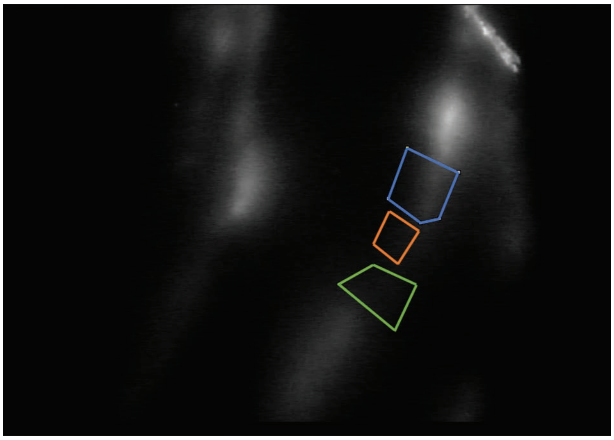

Fig. 2. Multiple regions of interest, taken on the same lymphatic vessel, confirm signal intensity curves.

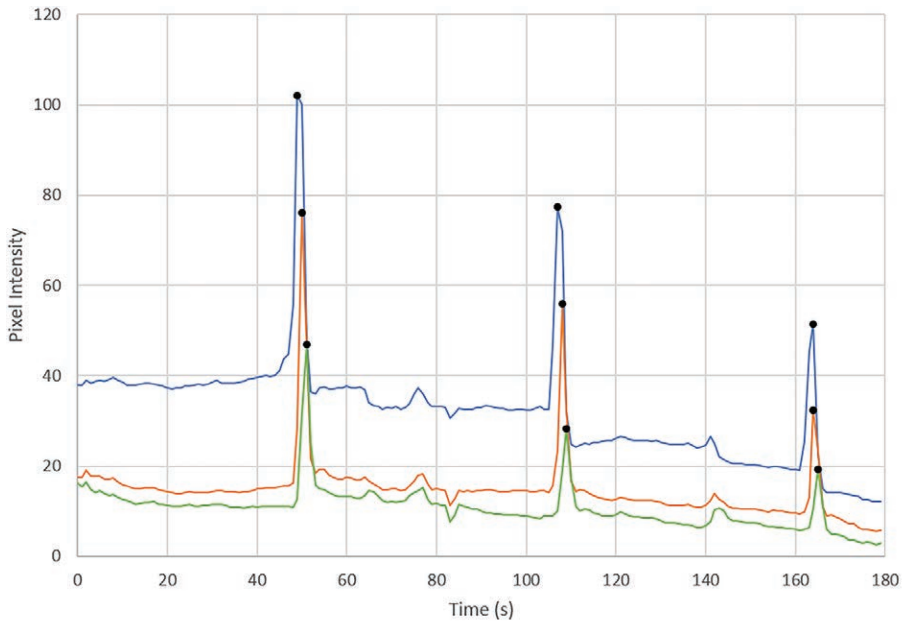

Fig. 3. Reproducibility of the signal intensity curve is depicted. The reproducibility of multiple regions of interest demonstrates that peaks captured are a product of a lymph bolus moving through the vessel, and are not noise or exposure variations being interpreted as peaks.

using a 3-minute, standard-of-care video capture. In our patient cohort, the average lymphatic contractility rate was 1.13 contractions per minute (range, 0.67 to 2.5 contractions per minute). A previous quantification technique using a custom-built near-infrared imaging system found propulsive events to occur every 42.1 ± 31.4 seconds in an upper extremity without lymphedema symptoms.[7] This corresponds to 1.4 contractions per minute, which falls within the range in our patient cohort of 1.13 contractions per minute (range, 0.67 to 2.5 contractions per minute).[7] In previous studies, contractility was calculated for the entire extremity. We were unable to conduct an analogous analysis, as we used only standard-of-care lymphangiograms of the dorsum of hand of the extremity of intervention.

A limitation of this methodology is that the region of interest must be selected by hand using visual judgment within the parameters previously defined, which may introduce unintentional bias. This was limited with a second, confirmatory region of interest on each channel. In the future, we will explore automatically selected regions of interest. Our study is also limited by the number of videos available for review. We will continue to investigate and substantiate our approach in a larger sample of videos. Finally, as we are using standard-of-care lymphangiograms, we do not have data from other anatomical locations along the extremity which, along with a larger sample size and more diverse patient population, including those with chronic lymphedema, would be necessary to establish a normal reference range.

In a field seeking further validation, objective measures of lymphatic function will be critical for establishing parameters to assess a patient's lymphatic function. Our proposed technique uses hardware already widely in use as the standard-of-care and requires 3 minutes for data acquisition. Contractility rate has the potential to serve as a clinically useful biomarker for disease status, allowing for surveillance of patients at high risk for the development of lymphedema, with potential for earlier therapeutic intervention, and monitoring of those already afflicted with the disease. Moreover, this methodology provides a potential postoperative objective measure that may help differentiate and substantiate various methods of immediate and delayed lymphatic reconstruction.

Dhruv Singhal, M.D.
Division of Plastic and Reconstructive Surgery
Beth Israel Deaconess Department of Surgery
110 Francis Street, Suite 5A
Boston, Mass. 02215
dsinghal@bidmc.harvard.edu
Twitter: @drdhruvsinghal

REFERENCES

1. Narushima M, Yamamoto T, Ogata F, Yoshimatsu H, Mihara M, Koshima I. Indocyanine green lymphography findings in limb lymphedema. *J Reconstr Microsurg.* 2016;32:72–79.
2. Rasmussen JC, Tan IC, Marshall MV, et al. Human lymphatic architecture and dynamic transport imaged using near-infrared fluorescence. *Transl Oncol.* 2010;3:362–372.
3. Ogata F, Narushima M, Mihara M, Azuma R, Morimoto Y, Koshima I. Intraoperative lymphography using indocyanine green dye for near-infrared fluorescence labeling in lymphedema. *Ann Plast Surg.* 2007;59:180–184.
4. Marshall MV, Rasmussen JC, Tan IC, et al. Near-infrared fluorescence imaging in humans with indocyanine green: A review and update. *Open Surg Oncol J.* 2010;2:12–25.
5. Unno N, Nishiyama M, Suzuki M, et al. Quantitative lymph imaging for assessment of lymph function using indocyanine green fluorescence lymphography. *Eur J Vasc Endovasc Surg.* 2008;36:230–236.
6. Suzuki M, Unno N, Yamamoto N, et al. Impaired lymphatic function recovered after great saphenous vein stripping in patients with varicose vein: Venodynamic and lymphodynamic results. *J Vasc Surg.* 2009;50:1085–1091.
7. Tan IC, Maus EA, Rasmussen JC, et al. Assessment of lymphatic contractile function after manual lymphatic drainage using near-infrared fluorescence imaging. *Arch Phys Med Rehabil.* 2011;92:756–764.e1.
8. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap): A metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform.* 2009;42:377–381.
9. Johnson AR, Singhal D. Immediate lymphatic reconstruction. *J Surg Oncol.* 2018;118:750–757.