

Randomized Feasibility Study Evaluating Multiple FDA-approved Microbubbles for CEUS Lymphography

Samuel Jang, MD*
 Jess D. Rames, MD, ME†
 Gina K. Hesley, MD*
 Nathan J. Brinkman, PharmD,
 RPh‡
 Nho V. Tran, MD‡
 Vahe Fahradyan, MD‡
 Christine U. Lee, MD, PhD*

Summary: Prior studies on contrast-enhanced ultrasound (CEUS) lymphography for preoperative mapping before lymphaticovenous anastomosis surgery in patients with extremity lymphedema have been limited to using only Lumason and Sonazoid as microbubble contrast agents. The purpose of this study was to determine the feasibility of using two other Food & Drug Administration–approved microbubble agents, Optison and Definity, for imaging lymphatic vessels in the upper extremities. Nine female adults with unilateral upper extremity lymphedema anticipating lymphaticovenous anastomosis surgery underwent CEUS lymphography of the unaffected upper extremity randomized to either Lumason, Definity, or Optison. Lymphatic vessels were visualized in all but one case when undiluted Definity was used. In the eight upper extremities where lymphatic vessels were visualized, an average of eight intradermal injections of microbubbles were performed in the extremity. Lymphatic vessels could be identified in 57% (36 of 63) of the injections. The effective dilution for each of the microbubble agents is provided. This was the first successful demonstration of lymphatic vessel visualization using either Definity or Optison. Broadening the range of available microbubble agents for CEUS lymphography could improve accessibility to the procedure and provide potentially safer alternatives. (*Plast Reconstr Surg Glob Open* 2024; 12:e5985; doi: 10.1097/GOX.0000000000005985; Published online 17 July 2024.)

INTRODUCTION

Contrast-enhanced ultrasound (CEUS) lymphography of the upper extremities¹ is often performed before lymphaticovenous anastomosis (LVA) surgery² to help guide bypass site selection. The lymphatic system's ability to uptake intradermally injected microbubbles was first demonstrated in a swine model with melanoma,³ and later demonstrated in humans in the context of identifying sentinel lymph nodes of breast cancer.⁴ The gas within the microbubbles reflects the acoustic ultrasound energy because of the impedance mismatch between the bubbles and surrounding tissues, appearing echogenic under ultrasound.⁵ Sulfur hexafluoride lipid-type A microspheres (Lumason/SonoVue, Bracco Suisse SA) are most frequently used in the United States.⁶ In other

countries, lipid-encapsulated perfluorobutane microbubbles (Sonazoid, GE HealthCare) are popular, but they are not Food & Drug Administration (FDA)–approved.^{7,8} Broadening the range of commercially available microbubbles for CEUS lymphography would benefit plastic surgeons outside of the United States, as the procedure is performed by plastic surgeons without the involvement of radiologists. This study determines the feasibility of two other FDA-approved intravenous microbubble agents for visualizing lymphatic vessels in the upper extremity: perflutren lipid microspheres (Definity/Luminess, Lantheus) and perflutren protein-type A microspheres (Optison, GE HealthCare).

MATERIALS AND METHODS

We performed a single-center prospective randomized study after appropriate institutional review board approval. The study was compliant with the Health Insurance Portability and Accountability Act.

From the *Department of Radiology, Mayo Clinic, Rochester, Minn.; †Department of Plastic Surgery, Mayo Clinic, Rochester, Minn.; and ‡Department of Pharmacy, Mayo Clinic, Rochester, Minn.

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Intradermal administration of the microbubbles was exempt from investigational new drug application regulation by the FDA. Female adults with unilateral upper extremity lymphedema secondary to breast cancer and anticipating LVA surgery were enrolled from a separate study (NCT 05613946). Nine participants were randomized to receive Lumason, Definity, or Optison in a 1:1:1 ratio for CEUS lymphography of the contralateral normal extremity during an enrollment period from January to August of 2023 following written informed consent.

Lumason (5.0 mL) was prepared according to manufacturer instructions and used without dilution. Definity solution (1.5 mL), first activated using a Vialmix, was used without dilution for the first participant randomized to Definity; subsequent Definity participants received diluted Definity (1.5 mL of activated Definity diluted to a total 10 mL with preservative-free saline). Optison solution (3.0 mL) was diluted to a total of 10 mL with preservative-free saline before administration.

CEUS lymphography was performed in the upper extremity unaffected by lymphedema. A 25-gauge needle was used to inject microbubble suspension aliquots of 0.3–0.4 mL into various locations including the interdigital web spaces and the forearm, adapting to variable locations due to vascular access tubings and blood pressure cuffs. After forming a wheal, the distended skin was massaged firmly. Immediate CEUS lymphography was performed using a ML6-15 (4.5–15 MHz) transducer on a GE Logiq E9 scanner (General Electric Healthcare, Wauwatosa, Wisc.), as described previously.⁹ A radiology fellow and a radiologist with 22 years of experience performing US-guided procedures and interpreting CEUS images reviewed the images. We summarized our findings using descriptive statistics.

RESULTS

All participants were women [mean age, 51 years ± 11 (SD); range 42–75 years]. Lymphatic vessels could be visualized in eight of nine upper extremities [3 of 3 (100%) for Lumason, 2 of 3 (67%) for Definity, and 3 of 3 (100%) for Optison (Fig. 1)]. No lymphatic vessels could be detected in the single patient receiving Definity without dilution secondary to marked fascial plane and subcutaneous tissue enhancement, which was not seen in the subsequent two participants given diluted Definity. (See figure, Supplemental Digital Content 1, which displays contrast-enhanced ultrasound images showing marked fascial enhancement, showing that lymphatic vessels were not visualized in one patient who received Definity without dilution. Diluting Definity, which comes in 1.5 mL solutions, to a total of 10 mL using preservative-free saline was effective for CEUS lymphography. <http://links.lww.com/PRSGO/D350>.) Lymphatic vessels could be seen in 48% (12 of 25) of injections with Lumason, in 63% (10 of 16) with diluted Definity, and in 64% (14 of 22) with diluted Optison. Table 1 shows the microbubble agents injected, the dilution of the microbubble solution, and the rate of lymphatic vessel visualization for each participant. No adverse reactions related to microbubble injections occurred.

Takeaways

Question: Contrast-enhanced ultrasound lymphography with Definity/Lumason and Optison is performed for the first time to broaden the range of available microbubbles for improved accessibility to contrast-enhanced ultrasound lymphography and to provide a potentially safer alternative to Lumason/SonoVue.

Findings: A small randomized study demonstrates that lymphatic channels can be visualized in the extremities with two additional commercially available microbubbles: Definity and Optison. The appropriate dilutions are provided.

Meaning: Ultrasound with intradermal injections of Definity and Optison, in addition to Lumason and Sonazoid, can identify lymphatic vessels for lymphaticovenous anastomosis surgery in the extremities.

DISCUSSION

CEUS lymphography with the three FDA-approved microbubble contrast agents effectively visualized superficial lymphatic vessels in the upper extremities. This was the first successful demonstration of lymphatic vessel visualization using either Definity or Optison. As an adjuvant to indocyanine green lymphography, CEUS lymphography has proven advantageous in identifying anastomotic surgical sites missed by conventional imaging techniques.^{6,7} Given the rare but increasing rate of severe adverse drug reactions associated with intravenous injection of Lumason,⁶ broadening the range of available microbubble agents for CEUS lymphography could improve accessibility to performing the procedure and provide potentially safer alternatives for providers.

Our experience has shown that a lymphatic vessel is not always detected with every microbubble injection. Lymphatic vessels could be seen with 57% (36 of 63) of the injections in this study. A prior study of CEUS lymphography using Sonazoid on the upper extremities of healthy participants reported a success rate of 98%. However, the injection was repeated up to three times at nearby skin site if no lymphatic vessels were seen, at the expense of additional injections, time, and microbubbles. As the study noted, ensuring intradermal placement of the microbubble bolus and massaging the skin wheal can increase the success rate. The effectiveness of each injection can also be affected by the homogeneity of the microbubble suspension and the injection volume. Specially designed needles for more efficient intradermal injections to improve lymphography in multiple modalities are currently under investigation.

Limitations of the study include the small sample size as a feasibility study. However, the high success rate of visualizing lymphatic vessels with each microbubble agent is promising. A larger trial comparing the diagnostic efficacy of the different microbubble agents could be worthwhile. Additionally, this study evaluated the upper extremities unaffected by lymphedema because the contralateral upper extremities with lymphedema underwent

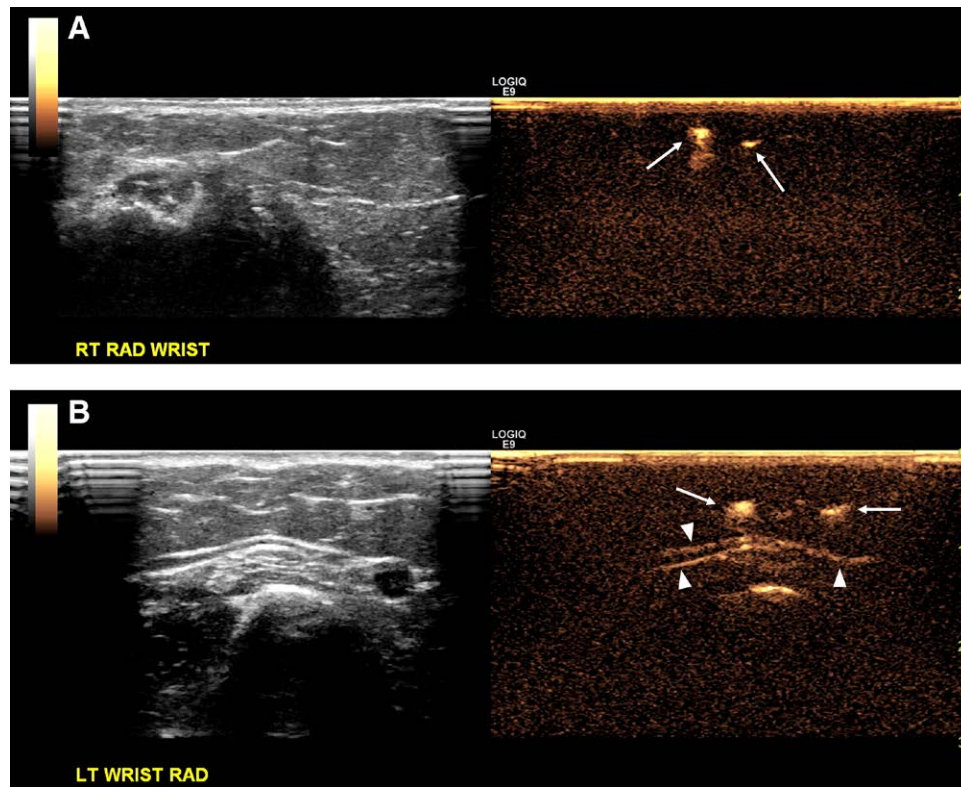


Fig. 1. Contrast-enhanced ultrasound images showing uptake of microbubbles in lymphatic vessels. Diluted Optison (A) and diluted Definity (B) were injected intradermally into the radial aspect of the wrists. Dual-screen display of the gray-scale and contrast-enhanced ultrasound images with the ultrasound transducer held perpendicular to the long axis of the arm shows uptake of microbubbles by lymphatic vessels (arrows). Fascial planes are also artifactually echogenic on CEUS (arrow heads), which can be discriminated from microbubbles in lymphatic vessels by comparing to the gray-scale images.

Table 1. Participants’ Randomized Microbubble Agents, Dilutions, and Outcomes

Patient	Age (y)	Microbubble Agent	Volume of Total Preparation (mL)	Laterality	No. Injections	No. Lymphatic Vessels Seen
A	71	Lumason*	5	Left	10	6
B	63	Lumason	5	Left	7	3
C	69	Lumason	5	Right	8	3
D	75	Definity†	1.5	Left	5	0
E	46	Definity	10	Left	9	5
F	41	Definity	10	Left	7	5
G	64	Optison‡	10	Right	9	5
H	59	Optison	10	Left	8	6
I	55	Optison	10	Right	5	3

*Preparation of a Lumason kit according to manufacture instruction yields a 5.0mL microbubble suspension, of which practically 4.8mL can be withdrawn.

†Definity is supplied as a 1.5 mL solution, which is first activated by Vialmix.

‡Optison is supplied as a 3.0 mL solution. It is the only microbubble without polyethylene glycol.

concurrent clinical CEUS lymphography with Lumason in preparation for LVA surgery. Prior reports of successful CEUS lymphography in extremities both affected and unaffected by lymphedema^{6,7} suggest that the procedure is effective regardless of lymphedema status.

Christine U. Lee, MD, PhD
 Mayo Clinic
 200 First St, SW
 Rochester, MN 55920
 E-mail: lee.christine@mayo.edu

DISCLOSURES

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