Fluorescein Isothiocyanate

A Novel Application for Lymphatic Surgery

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Abstract: The Lymphatic Microsurgical Preventing Healing Approach (LYMPHA) procedure entails performing a lymphovenous bypass (LVB) at the time of axillary lymph node dissection to reduce lymphedema risk. The two most common fluorophores utilized in LVB are blue dye and indocyanine green. We developed a novel application of fluorescein isothiocyanate for intraoperative lymphatic mapping. Our goal is to demonstrate the safety and efficacy of fluorescein isothiocyanate for this application. We reviewed a prospectively collected database on breast cancer patients who underwent LYMPHA from March to September 2015. Fluorescein isothiocyanate was used to identify arm lymphatic channels after axillary lymph node dissection to perform an LVB between disrupted lymphatics and axillary vein tributaries. Data on preoperative and intraoperative variables were analyzed. Thirteen patients underwent LYMPHA with intraoperative fluorescein isothiocyanate lymphatic mapping from March to September 2015. Average patient age was 50 years with a mean body mass index of 28. On average, 3.4 lacerated lymphatic channels were identified at an average distance of 2.72 cm (range, 0.25–5 cm) caudal to the axillary vein. On average, 1.7 channels were bypassed per patient. Eleven anastomoses were performed to the accessory branch of the axillary vein and 1 to a lateral branch. In 1 patient, a bypass was not performed due to poor lymphatic caliber and inadequate length of the harvested vein tributary. No intraoperative adverse events were noted. Fluorescein isothiocyanate is a safe and effective method for intra-operative lymphatic mapping. Fluorescein isothiocyanate imaging allows for simultaneous dissection and lymphatic visualization, making it an ideal agent for lymphatic mapping and dissection in open surgical fields, such as in the LYMPHA procedure.

Key Words: FITC, LYMPHA, lymphatic surgery

(*Ann Plast Surg* 2017;78: S296–S298)

In 2009, Dr. Campisi introduced a technique to prevent lymphedema termed the Lymphatic Microsurgical Preventing Healing Approach (LYMPHA).[1] In this technique, lymphatics draining the arm are identified and bypassed into an axillary vein tributary at the time of an axillary dissection. Dr. Campisi's team has reported an unprecedented 5% lymphedema rate after axillary lymph node dissection (ALND) and LYMPHA over a four year follow-up.[2] Historical rates of lymphedema after ALND are highly variable however usually are quoted between 20% and 40%[3–6] and have been reported as high as 77%.[7] The challenge of the LYMPHA procedure is visualizing healthy cut lymphatics lateral to the level 1 lymph nodes after an ALND. Dr. Campisi's team was able to identify these lymphatics by injecting blue dye into the ipsilateral proximal upper arm. However, breast surgeons often prefer to use a dual tracer method including both blue dye and technetium sulfur colloid for sentinel lymph node (SLN) identification. This is especially important in cases where neoadjuvant chemotherapy has previously been administered.[8,9] Therefore, a different dye was sought for arm lymphatic mapping to differentiate staining from arm versus breast lymphatics.

The most common method of lymphatic vessel mapping currently in use is indocyanine green (ICG). However, the challenge with ICG is that the dye is near-infrared and therefore excited in the nonvisible spectrum. This limits the usefulness of ICG for visualization and simultaneous dissection because the dye is displayed as a white signal on a black background and cannot be concurrently visualized through the binoculars of a microscope (Fig. 1A). Fluorescein isothiocyanate (FITC), on the other hand, is excited in the visible spectrum and routinely used in the operating room. Neurosurgeons inject this dye intravenously and use microscopes equipped with filter technology to visualize tumors while maintaining life-like color of the surrounding tissues allowing for simultaneous magnification and tissue dissection (Fig. 1B). This is a powerful property for the lymphatic surgeon. Although FITC has been used outside the operating room for lymphatic mapping in the skin,[11–13] to our knowledge, it had never been used in the operating room for lymphatic mapping. More recently, a single case report was published from France where FITC was used to perform a lymphovenous bypass (LVB) in the superficial tissues of the arm in a patient with chronic lymphedema.[14]

We hypothesized that FITC would be a safe and highly effective dye for lymphatic mapping and dissection in open surgical fields, such as in the LYMPHA procedure.

METHODS

We reviewed our prospectively collected Lymphedema Repository data on all breast cancer patients who underwent the LYMPHA procedure from March to September 2015. All patients that underwent the LYMPHA procedure with FITC were included in the review, and the charts were reviewed for demographic information (age, body mass index) and perioperative data (number of lymphatic channels visualized and bypassed, distance of channels from axillary vein, name of targeted vein, and adverse events).

Surgical Technique

Immediately before the ALND, 2 mL of a modified 2% fluorescein solution are injected intradermally and along the muscle fascia of the ipsilateral upper arm (Fig. 2A). Our solution is modified from the stock AK-FLUOR 10% (Akorn Inc, Lake Forest, Ill) solution by diluting 2 cc with 7.5 cc of normal saline and 0.5 cc of AlbuRx5 (CSL Behring Inc, King of Prussia, Pa). The ALND is performed with careful attention to preserve a superficial accessory vein tributary which

Received December 13, 2016, and accepted for publication, after revision December 24, 2016.

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Conflicts of interest and sources of funding: none declared.

Presented at the Plastic Surgery Research Council (New York, NY), May, 2016.

Presented at the Southeastern Society for Plastic and Reconstructive Surgeons, June, 2016.

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ISSN: 0148-7043/17/7806–S296

DOI: 10.1097/SAP.0000000000001034

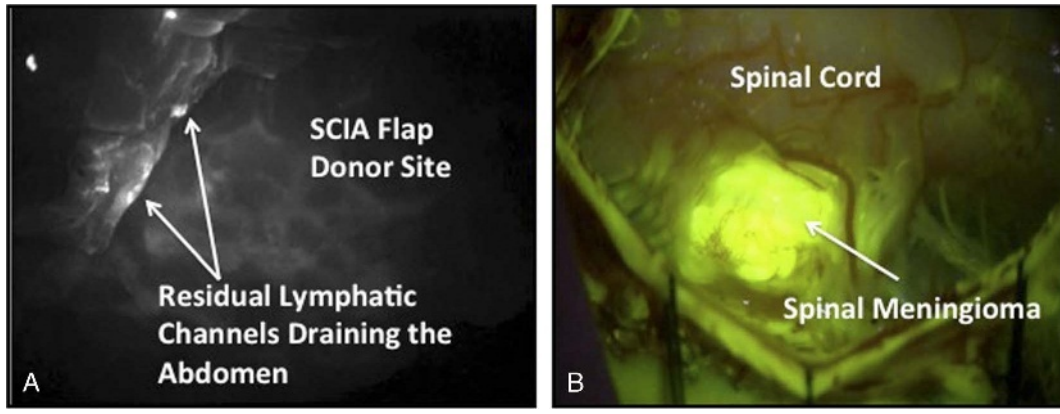

FIGURE 1. Comparison of ICG and FITC Imaging. A, Image of an superficial circumflex iliac artery donor flap harvest site with reverse lymphatic mapping[10] of the abdomen with ICG. Residual lymphatics draining the abdomen are identified as a white signal on a black background. Of note, despite the microscope being equipped with ICG capabilities, this image cannot be visualized in real-time through the binoculars and instead is displayed on a secondary screen. B, FITC imaging demonstrating a spinal meningioma. Note the life like color of the surrounding tissues and ability to visualize the target through the binoculars of the microscope allowing for simultaneous magnification and dissection of the meningioma.(Figure 1B courtesy of Dr. Maryam Rahman, Neurosurgery, UF Health).

longitudinally traverses the level 1 lymph nodes. The superior dissection of the level 1 axillary contents along the axillary vein is performed with identification of the accessory vein tributary which is typically found anterior to the thoracodorsal neurovascular bundle. The vein is then dissected free from the level 1 axillary contents and clipped distally to provide maximal length. Routine completion of the levels 1 and 2 ALND is then performed.

After completion of the axillary dissection, a Pentero 900D Microscope (Carl Zeiss Inc, Germany) equipped with the YELLOW 560 package is used to identify and map the cut lymphatic channels draining the arm. The harvested vein is prepared per standard microsurgical technique. Using 9–0 nylon suture, a "U" stitch is placed to capture the anterior wall of the vein and parachute in the lymphatic channels chosen for bypass.[1] (Fig. 2B) 10-0 nylon is then used to suture the

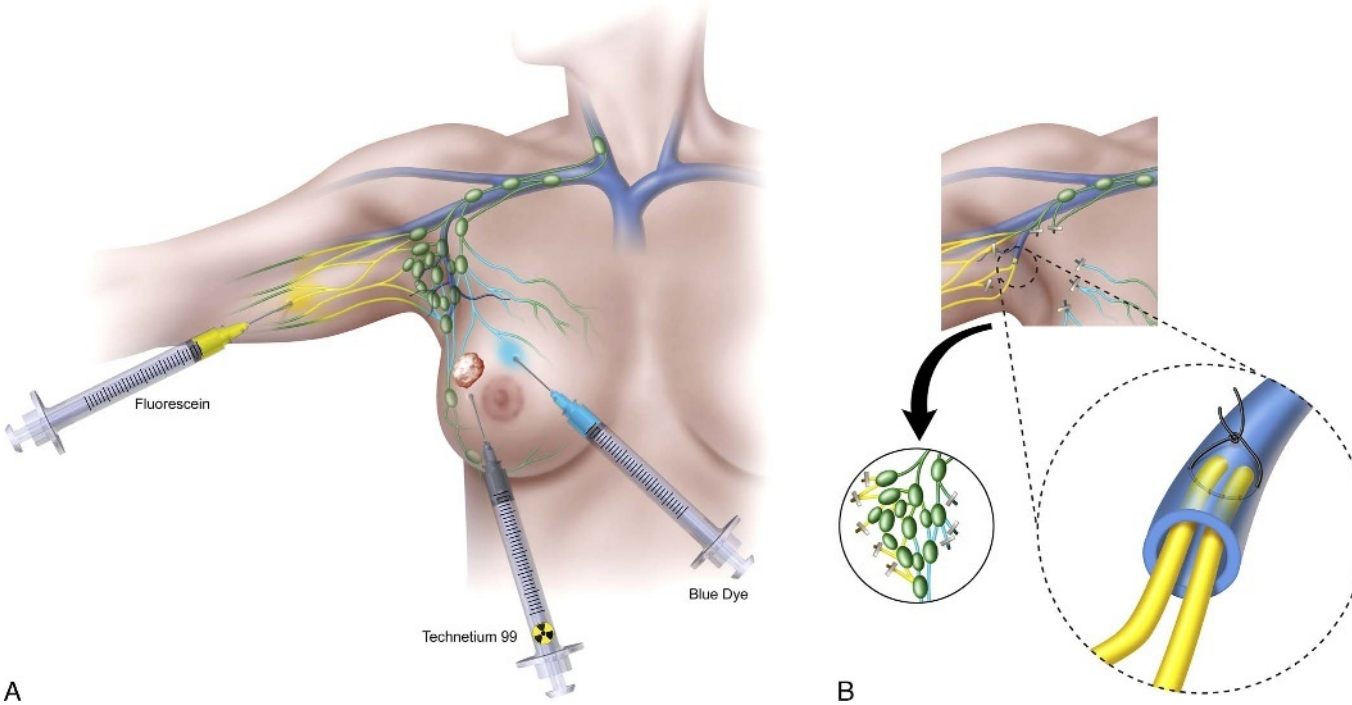

FIGURE 2. Schematic of the modified LYMPHA technique using FITC. A, Both blue and nuclear dyes are reserved for breast sentinel lymph node identification. FITC is injected in the proximal upper inner arm. B, After completion of the axillary dissection and removal of levels 1 and 2 lymph nodes, arm lymphatic channels, now "glowing" from the FITC injection, are identified and re-routed into an axillary vein tributary.

TABLE 1. Advantages and Disadvantages of the 2 Most Commonly Used Fluorophores in Lymphatic Surgery (Blue Dye and ICG) in Comparison to FITC

Dye	Advantages	Disadvantages
Blue dye	Technical	Technical
	✔Visualized through binoculars (live surgery)	✗No depth of penetration
	✔No specialized equipment necessary	✗Permanent training
		Safety
		✗Adverse reactions
		Skin necrosis (methylene blue)
		Anaphylaxis (isosulfan blue)
		✗Cross-reactivity
		Sulfa drugs (isosulfan blue)
		SSRI (methylene blue)
ICG	Technical	Technical
	✔Depth of penetration = 20 mm	✗Unable to visualize through binoculars (no live surgery)
	Safety	✗Permanent staining
	✔No adverse reactions (dermal)	✗Requires specialized equipment
	✔No cross-reactivities	
FITC	Technical	Technical
	✔Visualized through binoculars (live surgery)	✗Requires specialized equipment
	✔Depth of penetration = 5 mm	
	✔No permanent staining	
	Safety	
	✔No adverse reactions (dermal)	
	✔No cross-reactivities	

SSRI, selective serotonin reuptake inhibitor.

wall of the vein to the perilymphatic tissue. Channels not bypassed are clipped. Lymphatic flow filling the vein can be visualized with the filter activated 1 hour after anastomosis. (Supplemental Digital Content 1, http://links.lww.com/SAP/A232. Video demonstrating the modified LYMPHA procedure with FITC for arm lymphatic visualization and confirmation of flow into the venous system).

RESULTS

Thirteen patients underwent LYMPHA with intraoperative FITC lymphatic imaging from March to September 2015. Average patient age was 50 years with a mean body mass index of 28. On average, 3.4 lacerated lymphatic channels (range, 1–8) were identified at an average distance of 2.72 cm (range, 0.25–5 cm) caudal to the axillary vein. 1.7 channels were bypassed per patient (0–4). Eleven anastomoses were performed to the accessory branch of the axillary vein and 1 to a lateral branch. In 1 patient, a bypass was not performed due to poor lymphatic caliber and inadequate length of the vein tributary. No intraoperative or 30-day postoperative adverse events were noted. LYMPHA added an average of 67 minutes (45–120 minutes) to the oncologic procedure.

DISCUSSION

This study demonstrates that FITC is a safe and effective dye for the LYMPHA technique. In comparison to ICG and blue dye, FITC has many advantages.(Table 1) Fluorescein isothiocyanate is a safe dye used commonly by our neurosurgery and ophthalmology colleagues with a high safety profile.[15–17] No serious adverse reaction has ever been documented, to our knowledge, when injected intradermally. Moreover, FITC does not permanently stain surrounding tissues, as opposed to ICG and blue dyes, which facilitates dissection of the lymphatic channels. The primary advantage of FITC over ICG in lymphatic surgery is the ability to allow for simultaneous visualization and dissection of lymphatic channels because FITC is excited in the visible spectrum, making it an ideal dye to be used in open surgical fields. The limitations of FITC are that its depth of penetration is a quarter that of ICG (therefore, ineffective for transdermal visualization), and it does require specialized equipment for visualization. However, as we noted at our institution, this specialized equipment if often already readily available.

CONCLUSIONS

Fluorescein isothiocyanate is a safe and effective technique for lymphatic mapping in the LYMPHA technique. We encourage our colleagues to explore the power of FITC in open surgical fields to simultaneously visualize, magnify, and dissect lymphatic channels.

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