The Mechanism of Vascularized Lymph Node Transfer for Lymphedema: Natural Lymphaticovenous Drainage

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Background: Vascularized lymph node flap transfer for the treatment of upper and lower limb lymphedema has had promising results. This study was performed to investigate the mechanism of lymph drainage of a vascularized lymph node flap both experimentally and clinically.
Methods: In the experimental study, 18 Sprague-Dawley rats were used to create 36 flaps, either a groin lymph node flap or an abdominal cutaneous flap that did not contain lymph nodes. Indocyanine green dye was injected into the edge of 12 lymph node flaps, directly into a lymph node of 12 lymph node flaps, and into the edge of 12 cutaneous flaps. In the clinical study, an identical study design was used, with 24 vascularized lymph node flaps and 12 cutaneous flaps not containing lymph nodes.
Results: Experimentally, fluorescence was detected in the pedicle vein after a mean latency period of 153 ± 129 seconds when the edge of the lymph node flap was injected and 12.8 ± 8.1 seconds when the lymph node was directly injected. Fluorescence was not detected in the pedicle vein of the cutaneous flaps ($p < 0.01$). Clinically, fluorescence was detected in the pedicle vein after a mean latency period of 346 ± 249 seconds when the edge of the lymph node flap was injected and 23.5 ± 27.1 seconds when the lymph node was directly injected. Fluorescence was not detected in the pedicle vein of the cutaneous flaps ($p < 0.01$).
Conclusion: The vascularized lymph node flap drains lymph into the pedicle vein, both experimentally and clinically. (*Plast. Reconstr. Surg.* 133: 192e, 2014.)
CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, V.

Lymphedema of the upper extremity is mostly a sequela of breast cancer treatment with mastectomy and radiation,[1,2] and thus is often referred to as breast cancer–related lymphedema. It is a morbidity that affects an estimated 6 to 30 percent of survivors[3,4] and has a severe impact on quality of life, as it interferes with most activities of daily living.[5] Risk factors include greater number of lymph nodes removed, larger size of primary tumor, exposure to multifield radiation, higher body mass index, and upper outer quadrant tumor site.[6] Lower extremity lymphedema is a side effect of pelvic surgery and radiotherapy for patients with uterine corpus and endometrial malignancies, and the incidence increases with the number of regional lymph nodes removed.[7–9]

As survivorship of these cancers increases, more clinicians are committed to awareness of these associated sequelae. Several surgical approaches have been introduced, including lymphaticovenous bypass[10–20]; attempts to reduce soft-tissue volume, such as liposuction,[21–23] partial excision, and the Charles procedure[24–27]; local latissimus dorsi myocutaneous flap transfer to the axilla[5,28,29]; free omental flap or abdominal flap transfer with or without lymph nodes to the axilla[26,29–32]; and vascularized groin lymph node transfer.[33–36] In 2009, Cheng et al.'s group reported

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a mean circumferential reduction rate of 50 ± 19 percent at 56 ± 27-month follow-up for the treatment of breast cancer–related lymphedema using vascularized groin lymph node transfer to the wrist.[36] Cheng et al.[37] invented a new vascularized submental lymph node flap based on the submental artery and including neck lymph nodes of level Ia and Ib, according to the Robbins classification for the treatment of lower limb lymphedema.[38] These vascularized lymph node flaps are reported to achieve a reduction of the limb circumference, a decrease in the number of cellulitis episodes, and an improvement in the tightness of the lymphedematous limb.[37] However, the mechanism of action of the vascularized lymph node flap, including the drainage of lymph inside the transferred flap, has not yet been firmly established.

Indocyanine green dye binds to large plasma proteins and emits fluorescence that can be detected by fluorescent imaging, enabling the evaluation of tissue perfusion, uptake, distribution, and clearance of dye-marked fluid.[39] It is widely utilized as an intravenous injection for optical arteriography and visualization of superficial vessels in several fields with a near-infrared source (energy, 0.16 W; wavelength, 780 nm).[33,40–44] Intradermal injection of indocyanine green dye has been confirmed as a safe method of real-time lymphatic mapping.[45] Depending on the fluorescence intensity and dosage of the dye, the penetration depth of the imaging is approximately 2 cm.[45] The identification rates of the dye for sentinel lymph node biopsy have been shown to be greater than 90 percent without allergic reactions.[46] Because of its record of safety and wide clinical acceptance, indocyanine green imaging was used intraoperatively to evaluate the mechanism of vascularized lymph node flaps both experimentally and clinically in this study.

METHODS

This study was approved by the institutional review board of Chang Gung Memorial Hospital. All animal procedures complied with the Chang Gung Memorial Hospital Animal Research Guidelines.

Experimental Study

Eighteen 3-month-old Sprague Dawley rats (Bio Lasco Taiwan Co. Ltd., Taipei, Taiwan) were used in the experimental study. The rats were kept at a temperature of 23°C with 50 percent humidity and 12:12-hour light-dark cycles, with free access to water and standard feed. During the procedure, the animals were anesthetized with isoflurane (Aesica Ltd., Queenborough, United Kingdom). Under a Leica M651 manual surgical microscope (Leica, Wetzlar, Germany) at 6× magnification, a flap with or without lymph nodes was dissected. A 0.05-ml aliquot of indocyanine green (Sigma Aldrich, St. Louis, Mo.) was then injected carefully into the flap edge or one lymph node, according to the experimental groupings described below. The small amount of 0.05 ml was chosen to prevent internal pressure damage to the lymph nodes. In a dark room, an infrared signal of 760-nm wavelength was activated by a custom-made device. The detector was a charge-coupled device camera (Sony HD Handycam CM05, 10.2 megapixels; Sony Corp., New York, N.Y.) that filters out wavelengths below 820 nm. The fluorescent signals were transcribed into a black-and-white image that was continuously observed on the camera screen for 15 minutes. The distribution pattern and the time to first detection of the dye in the pedicle vein (latency period) were recorded. After completion of the procedure, the animal was euthanized with an intracardiac injection of 2% lidocaine hydrochloride (U-Liang Pharmaceutical Co. Ltd., Taoyuan, Taiwan).

The flap raised in the rats was either a vascularized groin lymph node flap or a deep inferior epigastric perforator (DIEP) flap, a cutaneous flap containing no lymph nodes.[47,48] For the lymph node flaps, a 3 × 1.5-cm block of adipose and lymph nodes pedicled on the superficial circumflex iliac artery and vein was elevated from the bilateral groins. The pedicle was dissected up to its origin at the femoral vessels (Fig. 1, *above, left*). The lymph nodes were identified but not completely dissected from the adipose in order to preserve the lymphaticovenous connections (Fig. 1, *above, right*). A total of 24 flaps in 12 rats were elevated in this manner. An aliquot of 0.05 ml of indocyanine green was injected into each flap. In 12 flaps, the dye was injected into the lateral edge of the adipose, and great care was taken not to inject into a lymph node inadvertently. In the other 12 flaps, the dye was injected directly into a lymph node.

For the DIEP flaps, a rectangular incision was made on the abdomen beginning at the xiphoid, proceeding bilaterally along the costal margins, down laterally to the anterior superior iliac spine, and finally connecting across the lower abdomen. Beginning at the most cranial perforator, the anterior rectus fascia was opened and the perforator was traced intramuscularly to its source vessel

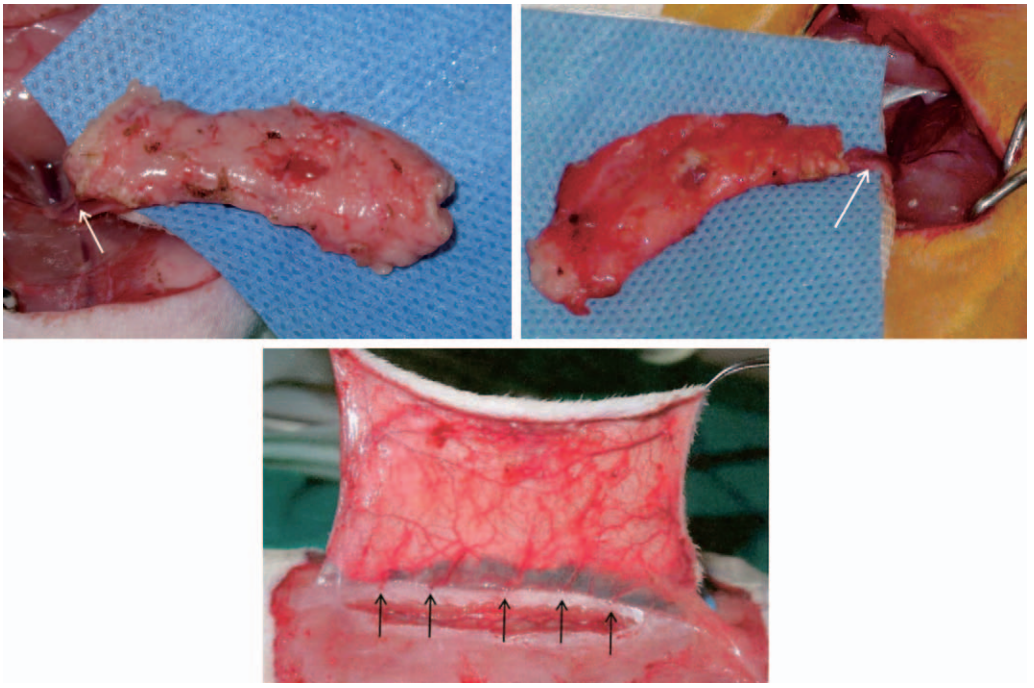

Fig. 1. The flaps of the experimental study. (*Above, left*) A left groin adipose lymph node flap (3 × 1.5 cm) pedicled on superficial iliac circumflex vessels (*white arrow*) was dissected out. (*Above, right*) The lymph node in the right groin adipose flap was partially exposed but not completely dissected to preserve the lymphaticovenous connections as the lymph node flap with node injection. The *white arrow* indicates the pedicle vein. (*Below*) The left DIEP flap was dissected with several visible perforators (*black arrows*), which were connected to superior epigastric vessels as a non–lymph node flap.

up to the costal margin. The pedicle vessels were then dissected caudally to where the most caudal perforators pierced the anterior rectus sheath (Fig. 1, *below*). Twelve flaps in six rats (one on each side of the longitudinal midline) were elevated in this manner. An aliquot of 0.05 ml of indocyanine green was injected into the subcutaneous tissue of the lateral flap edge.

Clinical Study

A parallel study design was used for the clinical study. Between January of 2012 and October of 2012, 24 patients who underwent vascularized lymph node flap transfer from the groin or submental area for upper or lower extremity lymphedema were included in this study. The protocol for harvesting these flaps was as previously described[36,37,49,50] and is shown in Figure 2, *above, left*. These flaps were injected after microvascular transfer. Also included in this study were 12 patients who in the same time period underwent a flap procedure involving a cutaneous flap not containing lymph nodes—namely, six forearm flaps and six anterolateral thigh flaps for head and neck cancer reconstruction. For practical reasons, these flaps were injected after they had been elevated, with the pedicle still in continuity at the donor site. As in the experimental study, 12 of the lymph node flaps were injected at the edge (not directly into a lymph node) (Fig. 2, *above, left*), 12 lymph node flaps were injected directly into a lymph node (Fig. 2, *above, right*), and 12 cutaneous flaps were injected at the edge (Fig. 2, *below*). For the clinical study, an aliquot of 1 ml was used for edge injections and 0.05 ml for lymph node injections. Drainage of the clinical flaps was observed for 30 minutes.

Statistical Analyses

The distribution pattern of indocyanine green was classified as follows: level 1, the injected dye stays local and shows no extension; level 2, the dye is diffused through less than half of the flap; level 3; diffusion involves the entire flap; and level 4, the dye drains directly into the pedicle vein without distributing within the flap. The distribution pattern of the dye and the time to first detection in the pedicle vein (latency period) were analyzed using SPSS 18.0 software (IBM Co., Armonk, N.Y.). Kruskal-Wallis and Mann-Whitney tests were utilized for time analyses. Statistical significance was defined as $p < 0.05$.

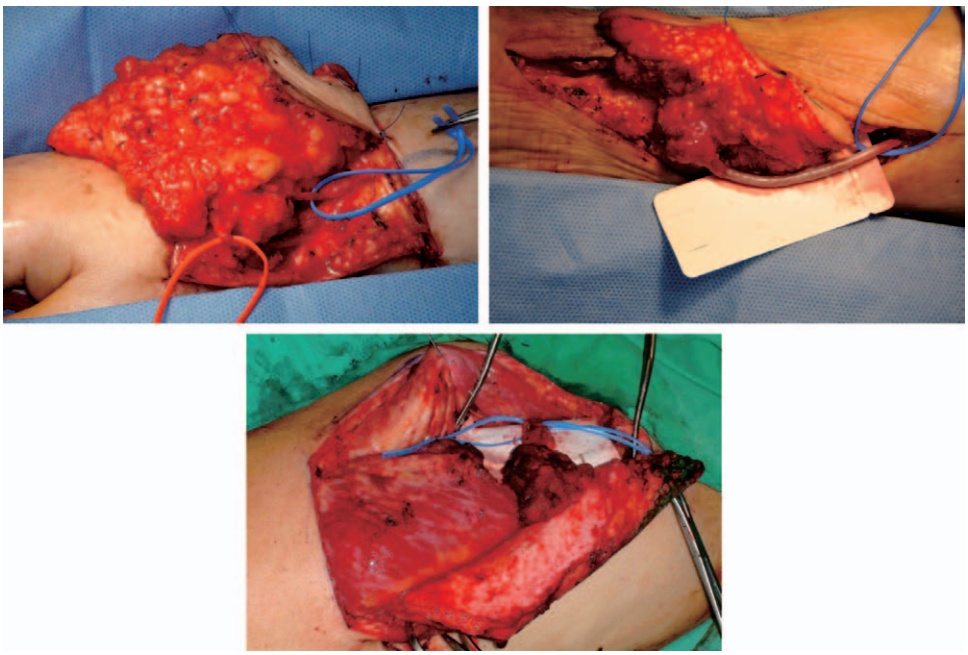

Fig. 2. The flaps of the clinical study. (*Above, left*) A vascularized groin lymph node flap was transferred to the right wrist. The donor vessels were anastomosed to the right radial artery dorsal branch (*red loop*) and cephalic vein (*blue loop*). (*Above, right*) A vascularized submental lymph node flap was transferred to the right ankle with donor vessels anastomosing to the posterior tibia artery and great saphenous vein (*blue loop*). (*Below*) A cutaneous anterolateral thigh flap was harvested with the pedicle in continuity (*blue loop*).

RESULTS

Experimental Study

The fluorescence drained into the pedicle vein in all lymph node flaps, after a mean latency period of 153 ± 129 seconds when the dye was injected into the edge of the flap (Fig. 3, *left*) and 12.8 ± 8.1 seconds when the dye was injected directly into a lymph node (Fig. 3, *center*). In contrast, no fluorescence was detected in the pedicle vein of the cutaneous with no lymph nodes (Fig. 3, *right*) ($p < 0.01$) (Table 1). The distribution pattern of fluorescence was level 4 in lymph node injections (i.e., no distribution in the flap itself due to direct drainage from the lymph node into the vein), level 3 (82 percent) or level 2 (18 percent) in edge injections of lymph node flaps, and level 1 in edge injections of non–lymph node flaps (Fig. 3).

Clinical Study

Parallel results were seen in the clinical study. All 36 flaps survived. Complications included an allergic reaction in one vascularized lymph node flap and venous insufficiency in six flaps, all of which were successfully salvaged. The fluorescence

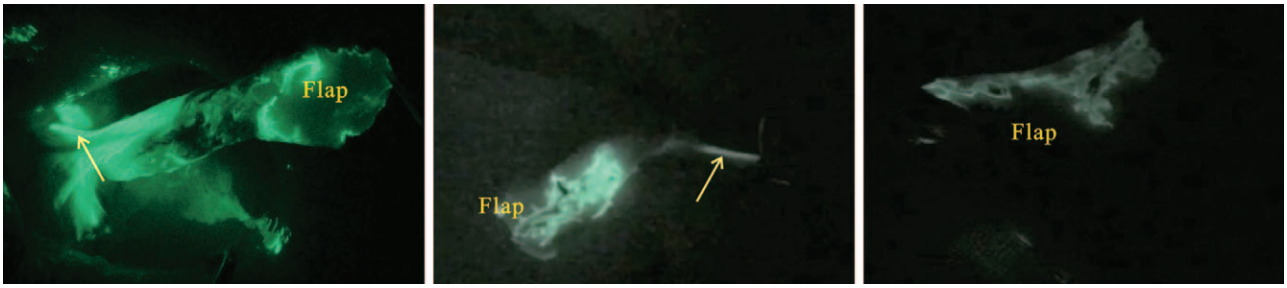

Fig. 3. Fluorescence distribution in the experimental study. (*Left*) The flap showed level 3 fluorescence distribution and drained into the pedicle vein (*arrow*) after injection of 0.05 ml of indocyanine green dye in the edge of the lymph node flap. (*Center*) The fluorescence rapidly and directly drained into the pedicle vein (*arrow*) after injection of 0.05 ml of dye into the lymph node. (*Right*) The fluorescence revealed level 1 distribution after injection of 0.05 ml of dye into the edge of the DIEP flap.

Table 1. Indocyanine Green Distribution Pattern and Fluorescence Time among Different Flaps in the Experimental and Clinical Studies

Flaps	No.	Fluorescence Distribution Pattern, *n* (%)				Mean Latency Period ± SD (sec)
		Level 1	Level 2	Level 3	Level 4	
Experimental study						
LN-flap edge injection	12		2 (16.7)	10 (83.3)		153.5 ± 129
LN flap node injection	12				12 (100)	12.8 ± 8.1
Non-LN flap injection	12	12 (100)				None
Total	36	12 (33.3)	2 (5.6)	10 (27.8)	12 (33.3)	83.1 ± 114*
Clinical study						
LN flap edge injection	12		1 (8.3)	11 (91.7)		346.5 ± 248.9
LN flap node injection	12				12 (100)	23.5 ± 27.1
Non-LN flap injection	12	6 (50)†		6 (100)‡		None
Total	36	6 (16.7)	1 (2.8)	17 (47.2)	12 (33.3)	185 ± 239.1*

LN, lymph node.
*$p < 0.01$.
†Anterolateral thigh flap.
‡Forearm flap.

drained into the pedicle vein in all lymph node flaps, after a mean latency period of 346 ± 249 seconds when the dye was injected into the edge of the flap (Fig. 4, *left*) and 23.5 ± 27.1 seconds when the dye was injected directly into a lymph node (Fig. 4, *center*). In contrast, no fluorescence was detected in the pedicle vein of the cutaneous flaps with no lymph nodes (Fig. 4, *right*) ($p < 0.01$) (Table 1). The fluorescence distribution pattern was level 4 in lymph node injections, level 3 (92 percent) or level 2 (8 percent) in edge injections of lymph node flaps, and level 1 (50 percent) or level 3 (50 percent) in edge injections of non–lymph node flaps (Fig. 4).

DISCUSSION

The transfer of vascularized lymph node flaps is a new and encouraging technique for the treatment of postoperative lymphedema of the upper and lower extremities. In this study, we endeavored to demonstrate the mechanism of lymph drainage in these flaps.

Initially, the animal part of the study was performed to confirm indocyanine green imaging as an appropriate technique for the evaluation of lymphatic drainage in these flaps. In demonstrating clearly visible and measurable drainage with fluorescence, the feasibility and reliability of this technique could be assured. More than this, however, the results of the experimental part of the study closely parallel and strongly corroborate the findings of the clinical study. In both parts, when the nodes themselves were injected, drainage into the pedicle vein was very rapid and direct, proving the existence and potency of lymphaticovenous connections in lymph node–containing tissue (Fig. 3, *center*, and Fig. 4, *center*). When the lymph node flaps were injected at the edge (i.e., not directly into a lymph node), drainage was slightly slower (Fig. 3, *left*, and Fig. 4, *left*), commensurate with the greater distance required for the dye to diffuse toward the lymph nodes, but it was still relatively rapid and direct, supporting the "pump" mechanism of vascularized lymph node flaps.[50] When non–lymph node–containing flaps

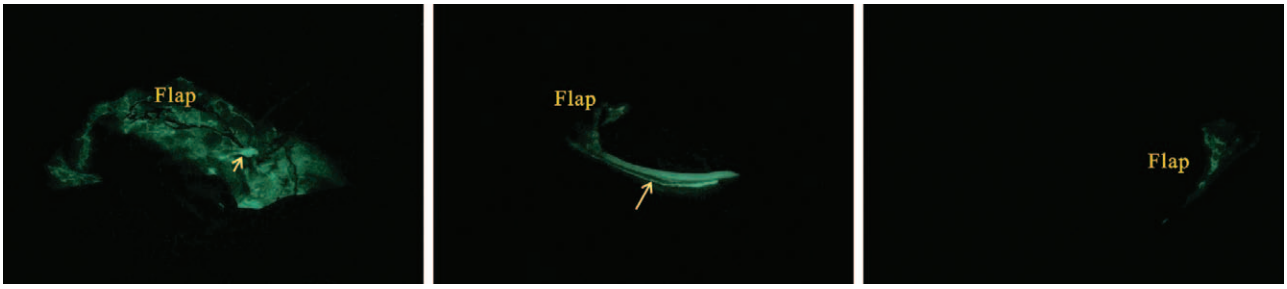

Fig. 4. Fluorescence distribution patterns in the clinical study. (*Left*) Fluorescence was demonstrated at level 3 and drained into the pedicle vein (*arrow*) after injection of 1 ml of indocyanine green dye into the flap edge. (*Center*) The fluorescence rapidly and directly drained into the pedicle vein (*arrow*) after injection of 0.05 ml of dye into the lymph node in the vascularized submental lymph node flap. (*Right*) The fluorescence stayed local at level 1 distribution after injection of 1 ml of dye into the edge of the cutaneous anterolateral thigh flap.

were injected, diffusion was slow and limited and no drainage was seen in the pedicle vein after a prolonged period of time (Fig. 3, *right*, and Fig. 4, *right*), proving that the subdermal venous plexus is not an important element in the mechanism of lymph drainage action. Interestingly, the forearm flap and the anterolateral thigh flap showed divergent distribution patterns, with the dye spreading out fairly quickly over the entire forearm flap (level 3 distribution) and remaining local in the anterolateral thigh flap (level 1 distribution) (Fig. 4 and Table 1). Presumably, the relatively rich subdermal plexus in the forearm skin compared with the thigh skin accounts for this difference.[51,52] Regardless of the reason, in neither flap did the dye reach the pedicle vein after 30 minutes, again supporting the lymph node as the crucial element in the lymph draining process.

CONCLUSION

The vascularized lymph node flap drains lymph from the interstitium to the lymph node and then into the pedicle vein.

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