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Interaction between vascularized lymph node transfer and recipient lymphatics after lymph node dissection- a pilot study in a canine model

Hiroo Suami, M.D., Ph.D.¹, Mario F. Scaglioni, M.D.¹, Katherine A. Dixon, R.T.², and Ramesh C. Tailor, Ph.D.³

¹Department of Plastic Surgery, The University of Texas MD Anderson Cancer Center, Houston, Texas

²Department of Interventional Radiology, The University of Texas MD Anderson Cancer Center, Houston, Texas

³Department of Radiation Physics, The University of Texas MD Anderson Cancer Center, Houston, Texas

Abstract

Background—Vascularized lymph node transfer (VLNT) has become more wide-spread for surgical treatment of lymphedema. However, interaction between a transferred lymph node and the recipient lymphatic system in relieving lymphedema has not been identified. The aims of this study were to investigate anatomical changes in the lymphatic system in the forelimb of a canine after lymph node dissection and irradiation and to clarify the interaction between the transferred lymph node and recipient lymphatics.

Materials and Methods—Two adult female mongrel canines were used for this exploratory study. The unilateral axillary and lower neck node dissections were performed, and 15-Gy irradiation was applied on postoperative day three. After one year, a VLNT flap was harvested

Corresponding Author: Hiroo Suami, M.D., Ph.D., Department of Plastic Surgery, Unit 1488, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030-4009, USA. Tel: +1-713-794-1247; fax: +1-713-794-5492; hsuami@hotmail.com.

Present Address: Hiroo Suami, M.D., Ph.D., Faculty of Medicine and Health Sciences, Bldg F10A, 2 Technology Place, Macquarie University, NSW 2109, Australia. Tel: +61-2-9812-3547; fax: +61-2-9812-3600; hiroo.suami@mq.edu.au

Authors' Roles:

Hiroo Suami (hsuami@hotmail.com): design of the study, performing animal procedures, collection of the data, imaging work, literature review, and writing of the manuscript.

Mario F. Scaglioni (mario.scaglioni@gmail.com): collection of the data, assisting design of the surgery, assisting with the surgeries and postoperative care, and editing the manuscript.

Katherine A. Dixon (Katherine.dixon@mdanderson.org): operating the C-arm and computed tomography, providing technical advice on animal imaging, collection of the imaging data, and editing the manuscript.

Ramesh C. Tailor (rtailor@mdanderson.org): calculating the dose of irradiation, providing technical advice on irradiation procedures, operating the irradiator, and editing the manuscript.

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from the lower abdominal region and inset in the axilla with vascular anastomoses. The girth of each forelimb was determined with a tape measure at different time points. Indocyanine green fluorescence lymphography and lymphangiography were performed before and after each surgery to evaluate morphological changes in the lymphatics.

Results—Both canines revealed identical changes in the lymphatic system but only one canine developed lymphedema. After lymph node dissection, a collateral lymphatic pathway formed a connection to the contralateral cervical node. After VLNT, an additional collateral pathway formed a connection to the internal mammary node via the transferred node in the axilla.

Conclusions—The findings suggest that the lymphatic system has a homing mechanism, which allows the severed lymphatic vessels to detect and connect to adjacent lymph nodes. VLNT may create new collateral pathways to relieve lymphedema.

Keywords

lymphatic system; lymph node dissection; lymphedema; canine; vascularized lymph node transfer; indocyanine green fluorescence lymphography; lymphangiography

INTRODUCTION

In developed nations, lymphedema is caused mainly by cancer treatment such as surgical ablation of regional lymph nodes and radiation therapy. The incidence of breast cancer-related lymphedema varies between reports, but is at least 20% in women who have undergone treatment for breast cancer.^{1,2} Despite the well-known morbidity of iatrogenic lymphedema, our knowledge of the pathophysiology of secondary lymphedema is very limited, and animal experiments are therefore desperately needed.³

Although little is known about the etiology of secondary lymphedema, surgical treatment of lymphedema has been attracting increasing attention from plastic surgeons. The current surgical options for lymphedema are roughly divided into two categories: ablative and physiologic.⁴ Vascularized lymph node transfer (VLNT), one of the physiologic procedures, is becoming more wide-spread and has produced promising outcomes.^{5–10} Two theories have been proposed to explain the relationship between VLNT and lymphedema. The first theory maintains that the transferred lymph node is a rich source of lymphangiogenic cytokines and facilitates bridging between the proximal and distal stumps of the lymphatic channels (bridging theory).^{8,9} The second theory posits that the lymph node possesses a native lymphovenous shunt and that a portion of lymph drains through the vascular pedicle, with the lymph node functioning like a suction pump (pumping theory).^{6,10}

Mouse models have been used to investigate lymphatic recanalization between a nonvascularized lymph node graft and recipient lymphatics by using the molecular biological approach.^{11,12} However, mice are not ideal for modeling VLNT in humans because their small size precludes vascular microanastomosis and tracing of lymphatic vessels.

We have investigated the comparative anatomy of the lymphatic system in different animals and humans.^{13–16} We believe that large-animal studies are imperative for understanding the

biological mechanisms of VLNT. We used canines in this exploratory study because the lymphatic system in the canine forelimb is similar to the lymphatic system in humans in terms of the arrangement of lymph nodes and clear separation between the superficial and deep components of the lymphatic system.^{13,15} The first aim of this study was to investigate anatomical alterations in the lymphatic system of the forelimb after radical lymph node dissection followed by irradiation of the surgical site. The second aim was to understand the interaction between the transferred vascularized lymph node and recipient-site lymphatics. We hypothesized that VLNT would facilitate collateral formation of the lymphatic system, thus VLNT would have the potential to treat lymphedema.

MATERIALS AND METHODS

All animal procedures were approved by The University of Texas MD Anderson Cancer Center's Institutional Animal Care and Use Committee, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International including the National Institutes of Health. Two adult female mongrel canines (canine A and canine B, weighing 25.4 and 23.0 kg respectively on arrival) were used. They underwent identical procedures (Fig. 1).

The left forelimb underwent all procedures, and the right forelimb served as a nonoperative control. A single examiner measured forelimb girth with a tape measure every 2–3 days during the first two weeks after each surgery and every 2–4 weeks thereafter. Girth was measured at the following four sites in both forelimbs with the animal in a standing position on an examination table: forepaw, wrist, elbow, and midlevel between the wrist and elbow.

Imaging before and after surgery

Indocyanine green (ICG) fluorescence lymphography (Photodynamic Eye; Hamamatsu Photonics K.K., Hamamatsu, Japan) and lymphangiography were used for imaging of lymphatic vessels and lymph nodes. After each canine was anesthetized with isoflurane gas and placed in the right lateral position, 0.2 ml of ICG (IC-Green, 2.5 mg/ml; Akorn, Lake Forrest, IL) was injected intradermally into the web space of each finger. Additional ICG injections were given at 5–7 spots along the borders of the lymphatic territories (lymphosomes)¹² for the preoperative examination. ICG flow in the lymphatic vessels was facilitated with gentle massage. Scanning with a handheld camera continued until the dye reached the corresponding lymph nodes. The lymphatic vessels were traced and the locations of the lymph nodes were marked with a skin marker. The ICG images were recorded in digital format.

After completion of the ICG fluorescence lymphography, lymphangiography was performed. The anesthetized canine was switched to the sternal position. Isosulfan blue (Lymphazurin, 0.2 ml; Covidien, Mansfield, MA) was injected intradermally into each web space. A 4-cm V-shaped skin incision was made in the left dorsal paw, and lymphatic vessels were identified and dissected under a surgical microscope. A small cannula with an outer diameter of 0.33 mm (Micro Cannulation System; Fine Science Tools Inc., Foster City, CA) was inserted into the lumen of a lymphatic vessel and secured with 8-0 nylon sutures. Oil-based radiocontrast medium (Lipiodol Ultra-Fluid; Guerbed, Roissy, France) was injected at

0.15 ml/min at the start and then increased to 0.5 ml/min with a syringe pump. Radiocontrast flow was scanned with a C-Arm (Mobile C-Arm Series 9800; OEC Medical Systems Inc., Salt Lake City, UT), and radiographic images were taken sequentially. The lymphangiography continued until the radiocontrast reached the first-tier (sentinel) nodes. After completion of the lymphangiography, the left forelimb and anterior upper torso were radiographed in the sternal and lateral positions using a portable digital X-ray system (RadPro; Canon USA Inc., Melville, NY). ICG fluorescence lymphography and lymphangiography were performed three times: before surgery, 6 months after the first operation, and 6 months after the second operation (Fig. 1).

Operation 1: Lymph Node Dissection and Irradiation

After the canines were anesthetized with isoflurane, ICG fluorescence lymphography was performed to identify the ventral and dorsal superficial cervical nodes and axillary nodes. A 25-cm C-shaped incision was made around the left axilla crossing over the three regional lymph nodes (Fig. 2). Isosulfan blue (0.2 ml) was injected intradermally into the distal forelimb and upper torso at several spots to stain the superficial and deep afferent lymphatic vessels in the left forelimb. After the skin incision, all three regional lymph nodes were identified, and the afferent and efferent lymphatic vessels and the nodes were resected together with subcutaneous fat tissue to create lymphatic gaps of the maximum distance. The distal and proximal stumps of each lymphatic vessel were ligated with 5-0 nylon sutures. The wound was closed with braided sutures in the dermal layer and skin staples.

The canines were anesthetized again on the third postoperative day, and the following field was irradiated on the left side of the body by using a cobalt-60 gamma irradiator (Theratron 780C; Theratronics, Ottawa, Canada): lower neck, shoulder, upper forelimb, and upper torso (Fig. 2). Tissue-equivalent bolus material (packaged rice) was placed over and under the upper arm to provide a build-up layer. To ensure dose uniformity, we placed the canines in both the sternal and the supine positions, and half of the dose was given in each position. Radiation was administered once at a total calculated dose of 15 Gy at a rate of 49–52 cGy/min.

Operation 2: Vascularized Lymph Node Flap Transfer

One year after the first surgery, the scar in the axillary portion was incised under general anesthesia. The recipient vessels were explored and thoracodorsal vessels or circumflex scapular vessels were dissected and secured. ICG fluorescence lymphography was performed in the left abdominal and medial thigh regions, and the afferent lymphatic vessels and the left inguinal lymph node were marked. An 18×7 cm fasciocutaneous flap encompassing the inguinal node was designed (Fig. 3). The vascularized lymph node flap was supplied by the direct cutaneous perforators: the superficial inferior epigastric artery and vein.¹⁷ The flap was elevated and inset in the left axilla (Fig. 4). The donor vessels were anastomosed with the recipient vessels using 9-0 nylon sutures under the surgical microscope. Each canine was kept in a small, quiet cage for 1 week after surgery and in a normal cage for another 1 week, and normal activities were resumed thereafter.

Terminal Procedures

One month after the post-VLNT imaging examinations, the canines were euthanized with an intravenous injection of Beuthanasia (0.25 ml/kg). The upper torso was radiographed in the sternal position and right lateral position. The upper torso of Canine B was scanned with helical computed tomography (LightSpeed; GE Healthcare, Milwaukee, WI).

The transferred inguinal lymph node in the axilla and the inguinal lymph node from the right (nonoperative) side were harvested, fixed with 10% formalin, sliced, and stained with hematoxylin and eosin for histological examination.

RESULTS

Canine A: manifestation of lymphedema

The preoperative imaging examinations revealed that the lymphatic vessels in the forelimb of canine A were connected to one ventral superficial cervical node and two axillary nodes (Fig. 5 left, Fig. 6 left, S1 and S2 Videos). During the lymph node dissection, all lymph nodes identified by lymphangiography were removed. On postoperative day 1, the forelimb became swollen. On postoperative day 14, the wound in the axillary portion was dehisced, and the raw surface healed by secondary intention. Swelling peaked 1.5 months after surgery (Fig. 7 left). The swelling in the upper arm gradually subsided; however the distal forearm maintained increased girth for 12 months (Fig. 7 middle, Fig. 8). Hence, we diagnosed development of lymphedema in the operated left forelimb.

Postoperative imaging was performed 6 months after surgery. ICG fluorescence lymphography demonstrated a dermal backflow pattern in the dorsal paw and distal forearm and around the operation scar (Fig. 5 middle, S3 Video). Many tortuous and large lymphatic vessels were observed in the medial forearm (Fig. 4). The lymphatic vessels crossed the midline and extended to the contralateral superficial ventral cervical lymph node, resembling a spider web. Lymphangiography produced similar findings, and sequential images demonstrated travel of the radiocontrast in the lymphatic vessels (Fig. 6 middle, S4 Video). The images initially showed a lymphatic vessel similar to that observed before surgery; however, the main trunk branched shortly after. The vessel filling patterns were not unidirectional from distal to proximal sites, and reflux was frequently observed suggesting valve incompetency in the lymphatic vessels. The swelling in the distal portion of the forelimb suggested that gravity contributed to the lymphedema. The contrast did not reach the scar before filling all lymphatic vessels in the forelimb. Three vials of radiocontrast medium (30 ml) were required for the examination compared to the one vial required before surgery.

The postoperative course of the VLNT was uneventful. The girth of the forelimb did not change initially but decreased slightly 6 months after surgery (Fig. 7 right, Fig. 8). Imaging at 6 months after VLNT revealed no further changes in the lymphatic system in the forelimb and collateral drainage to the contralateral cervical node. However, the ICG injected into the paw was taken up by the lymphatic vessels in the VLNT skin paddle and reached the transferred inguinal node in the axilla (Fig. 5 right, Fig. 6 right, S5 and S6 Videos). Of note, the efferent lymphatic vessel from the transferred node connected to the second- and third-

tier nodes inside the thoracic cavity. The lymphangiography findings proved that VLNT created a new collateral lymph pathway between the recipient lymphatic system in the forelimb and the intramammary lymph node.

The left hind limb showed no swelling after harvesting of the inguinal lymph node flap. ICG fluorescence lymphography showed that the lymphatic vessels originated from the left medial thigh, crossed the donor scar and abdominal midline, and connected to the right contralateral inguinal node.

Histological examination showed that the control inguinal lymph node had lymphoid follicles with germinal centers in the outer cortex and a medullary cord in the medulla. In contrast, the transferred inguinal node had diffuse vacuolar degeneration in both the cortex and the medulla (Fig. 9).

Canine B: no manifestation of lymphedema

The second canine underwent the same procedures as canine A. The postoperative course after the lymph node dissection was uneventful. The extent of swelling in the operated forelimb was moderate compared to canine A, and no significant change in forelimb in girth was observed 1 month after surgery.

Images obtained 6 months after lymph node dissection demonstrated that the lymphatic vessels in the forelimb maintained their original appearance from the dorsal paw until they reached the operated area. The dermal backflow pattern was seen around the operative scar, and four lymphatic vessels emerged proximal from the dermal backflow area, crossed the midline, and connected to the contralateral superficial ventral cervical node, as seen in canine A.

The post-VLNT course was uneventful. ICG fluorescence lymphography and lymphangiography 6 months after VLNT revealed that two collateral lymphatic pathways from the recipient forelimb connected to the contralateral ventral superficial cervical node and to the ipsilateral internal mammary node via the transferred inguinal node, as in canine A. Radiographs obtained as part of terminal procedures 1 month after lymphangiography demonstrated lymph nodes more clearly with remaining contrast media (Fig. 10). Computed tomography confirmed that the second-tier node relative to the transferred node was the ipsilateral internal mammary lymph node (Fig. 11). Histological examination of the transferred inguinal node demonstrated the same vacuolar degeneration pattern, as seen in canine A.

DISCUSSION

ICG fluorescence lymphography is used to noninvasively visualize lymphatic vessels^{18–20}. However, infra-red technology was limited in visualizing structures deeper than 1 cm below the surface. In contrast, lymphangiography required invasive procedures: skin incision and cannulation. The resolution of lymphangiography was superior to that of ICG lymphography, enabling the C-arm to trace deeper lymphatic structures. The disadvantages of the lymphangiography were radiation exposure and longer examination time.

Lymphangiography was used to investigate the pathogenesis of post-mastectomy lymphedema in the clinical setting. Abe classified lymphangiography images in three types: type 1, recanalization crossing the axilla (original pathway); type 2, recanalization through collateral pathways and type 3, blockage. He concluded that each type represented the degree of clinical lymphedema: mild, moderate and severe respectively²¹.

In our previous study, we found lymphatic alterations after lymph node dissection in a canine model without irradiation¹⁵. We observed collateral lymph formation from the surgical scar to the contralateral superficial cervical node in one of two canines. This finding is consistent with our current finding of the formation of a specific pattern of collateral lymph vessels that drained to the contralateral cervical lymph node after lymph node dissection in both canines.

We did not determine whether the collateral lymphatic vessels were newly created or whether they emerged from preexisting vessels, like “choke vessels” in the arterial system.²² However, we strongly suspect that these vessels were newly created, for the following reasons. The direction of lymph flow is regulated by valvular structures in the lumen of the lymphatic vessels, and intervals between valves are much shorter than those in veins.^{13,23} In our study, the direction of flow in the collateral lymph vessels was opposite to the original direction of flow between the scar and the front midline.

The ‘seed and soil hypothesis’, in which cancer cells (seed) get into the systemic circulation and settle down and grow in organs with favorable conditions (soil) has been widely accepted by oncologic scientists.^{24,25} However, clinical reports have described contralateral axillary metastasis after axillary lymph node dissection for breast cancer.^{26–29} This suggests that the seed and soil hypothesis may not be applicable to contralateral axillary lymph node metastasis. Our findings suggest that the formation of collateral lymphatic vessels and interaction between lymph nodes and lymphatic vessels after surgery play a key role in the initiation and development of distant metastases by local residual cancer in certain cases.

Two theories, the ‘bridging theory’ and ‘pumping theory’, have been proposed to explain the relationship between VLNT and lymphedema.^{6,8–10} Our results showed that the VLNT bridged the lymphatic surgical gap. However, the route was different from the original pathway, with the efferent lymphatic vessels from the VLNT connecting to the internal mammary lymph nodes in both canines. To the best of our knowledge, degeneration of a vascularized lymph node has not been previously reported. Temporary stagnation of lymphatic flow before establishment of lymph recanalization may have been the cause of this degeneration.

The findings of our study expand our understanding of the biological mechanism of VLNT. During this study, we saw reconnection of the severed lymphatic vessels to an adjacent node in each canine on four occasions; 1) the lymphatic vessels in the left forelimb connected to the contralateral cervical lymph node after operation 1, 2) the lymphatic vessels in the left forelimb connected to the transferred inguinal lymph node after operation 2, 3) the efferent lymphatic vessel from the transferred node connected to the ipsilateral intramammary lymph node, and 4) the lymphatic vessels in the left hind limb connected to the contralateral

inguinal lymph node after operation 2. The specific collateral patterns suggest that an unknown “lymph homing mechanism” may regulate recanalization of the severed afferent lymphatic channels to the adjacent lymph node. The lymph node appeared to serve as a “beacon” guiding the severed afferent lymphatic vessels.

Further investigation of the interaction between lymph nodes and severed lymphatic vessels can provide insights into ways of improving surgical treatment of lymphedema and can help increase our understanding of how the lymphatic system affects cancer development and local recurrence after lymph node dissection. It is likely that specific biomolecular mediators direct lymphangiogenesis toward a node, and bioinformatics analysis would be the next step in identifying such agents.

CONCLUSIONS

We observed a new lymphatic collateral pathway involving the transferred lymph node in our canine model. The lymph node appeared to serve as a biological beacon for lymphangiogenesis by guiding the severed lymphatic vessels toward itself. The interaction between the severed afferent lymphatic vessels and the lymph node suggests the existence of a lymph homing mechanism that locates an adjacent lymph node and stimulates recanalization toward the node. VLNT may help treat lymphedema by creating new lymphatic drainage pathways instead of bridging the original pathways.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
Experimental procedures.

Fig. 2.
Lymph node dissection. The incisions (red lines), lymph node dissection area (orange shading), and radiation field (yellow shading) are indicated.

Fig. 3. VLNT donor site (canine A) and tracing of lymphatic vessels and the inguinal lymph node (arrow) (left). ICG fluorescence lymphography in the same site (right).

Fig. 4. VLNT was inset in the axilla 3 weeks after surgery (canine A). Lymphatic vessels remained dilated in the medial forelimb.

Fig. 5. ICG fluorescence lymphographs of the medial forelimb and front chest of canine A before surgery (left), 6 months after lymph node dissection (middle), and 6 months after VLNT (right).

Fig. 6. Lateral (top) and anteroposterior (bottom) lymphangiographs of canine A before surgery (left), 6 months after lymph node dissection (middle), and 6 months after VLNT (right). The lymphatic vessels in the left forelimb connected to the contralateral superficial ventral cervical lymph node (red arrow), the transferred inguinal lymph node (green arrow), and the ipsilateral intramammary lymph node (yellow arrow).

Fig. 7.

Canine A 1.5 months after lymph node dissection and irradiation (left), after 12 months (middle), and 6 months after vascularized lymph node transfer. Remarkable swelling was seen in the operated forelimb after operation 1. The distal forearm maintained increased girth for 12 months. The girth of the forelimb decreased slightly 6 months after operation 2.

Fig. 8.

Temporal change in forelimb girth in canine A after lymph node dissection (Op1).

Red:operated-forelimb elbow; blue:nonoperated-forelimb elbow; purple:operated-forelimb wrist; green:nonoperated-forelimb wrist. Swelling peaked 1 month (M) after lymph node dissection. The swelling in the distal forelimb lasted 12 months.

Fig. 9.

Hematoxylin- and eosin-stained images of inguinal lymph nodes in canine A. An inguinal node from the control is shown on the left, and the transferred node in the axilla is shown on the right. Diffuse vacuolar degeneration can be seen in the transferred node. Scale bars, 5 mm.

Fig. 10.

A radiograph of canine B at the terminal procedures 1 month after lymphangiography, showing lymph nodes with remaining contrast media: the contralateral superficial ventral cervical node (red arrow), the transferred inguinal node (green arrow), and intramammary nodes (yellow arrows).

Fig. 11.

A computed tomography image of canine B showing the transferred node (green arrow) and an intramammary node (yellow arrow) enhanced by remaining radiocontrast media at the terminal procedures.