The Lymphatic Response to Injury with Soft-Tissue Reconstruction in High-Energy Open Tibial Fractures of the Lower Extremity

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Background: Severe compound tibial fractures are associated with extensive soft-tissue damage, resulting in disruption of lymphatic pathways that leave the patient at risk of developing chronic lymphedema. There are limited data on lymphatic response following lower limb trauma. Indocyanine green fluorescence lymphography is a novel, real-time imaging technique for superficial lymphatic mapping. The authors used this technique to image the superficial lymphatic vessels of the lower limbs in patients with severe compound tibial fracture.
Methods: Baseline demographics and clinical and operative details were recorded in a prospective cohort of 17 patients who had undergone bone and soft-tissue reconstruction after severe compound tibial fracture between 2009 and 2014. Normal lymphatic images were obtained from the patients' noninjured limbs as a control. In this way, the authors investigated any changes to the normal anatomy of the lymphatic system in the affected limbs.
Results: Of the 17 patients, eight had free muscle flaps with split-thickness skin grafting, one had a free fasciocutaneous flap, one had a full-thickness skin graft, six had local fasciocutaneous flaps, and one had a pedicled gastrocnemius flap. None of the free flaps demonstrated any functional lymphatic vessels; the fasciocutaneous flaps and the skin graft demonstrated impaired lymphatic vessel function and dermal backflow pattern similar to that in lymphedema. Local flaps demonstrated lymphatic blockage at the scar edge.
Conclusion: Severe compound fractures and the associated soft-tissue injury can result in significant lymphatic disruption and an increased risk for the development of chronic lymphedema. (*Plast. Reconstr. Surg.* 139: 483, 2017.)

One of the significant causes of lower extremity lymphedema is trauma, which can include iatrogenic insult that occurs with surgery.[1] Lymphedema is clinically defined as an abnormal degree of edema that persists beyond the period of acute injury and does not subside after 10 to 12 weeks. Although the range is variable, chronic lymphedema often manifests immediately after injury but may have an indolent onset, presenting months or even years after injury and apparent recovery. The lymphatic

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system exists in a delicate balance, and under normal conditions, a functional system can match transport capacity and lymphatic load. Over time, if the load presented is higher than the transport capacity, either because of additional lymphatic load or a damaged, attenuated lymphatic system, lymphedema will occur. In a lower limb that has suffered the multiple insults of trauma and attempts at reconstruction of bony and soft tissue, the transport capacity of lymph collectors is diminished. Our anecdotal experiences suggest that patients in this population will be at risk of developing lymphedema; however, the cause of the process remains poorly understood and consequently the time of onset and severity of disease is difficult to predict.

The soft-tissue injury associated with severe compound tibial fractures has the potential to cause significant soft-tissue damage and disruption to lymphatic vessels. Severe compound fractures have been categorized into the Gustilo classification. Type I and II fractures are clean and relatively small, soft-tissue wounds associated with fractures. Type III fractures involve significant amounts of soft-tissue loss.[2] Type III fractures are further subclassified into types A, B, and C according to periosteal stripping and vascularity. Complications such as infection and nonunion specifically following Gustilo type IIIB fracture reconstruction have been well documented.[2,3] However, the extent of damage to the lymphatic system and the subsequent risk of recurrent infections and chronic lymphedema remain relatively unstudied. Damage to soft tissue and stasis within the lymphatic system can lead to chronic lymphedema and add significant long-term morbidity to the injury.

Edema before the reconstruction or within the time frame of wound healing is a normal physiologic response to trauma. The presence of inflammation-related edema has been linked to lymphatic function, but the involvement of the lymphatic system has only been raised sporadically in research studies relating to soft-tissue injury.[4–6] As the wound healing progresses, there is unavoidable scar tissue formation that can further restrict lymphatic flow and impair function.[1,7]

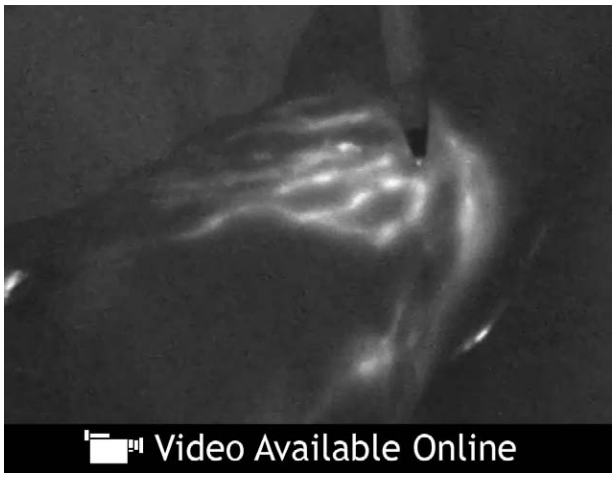
To date, limitations in the ability to perform minimally invasive, real-time lymphatic imaging underlies the paucity of information regarding lymphatic vessels and their response to trauma. The development of techniques using the fluorescence contrast agent indocyanine green with near-infrared imaging has allowed such visualization. Indocyanine green is a fast fluorescence-emitting contrast agent for lymphatics because of its ability to bind immediately to plasma proteins (mostly albumin) within the tissue spaces after intradermal injection. Also, its water-soluble properties, rapid excretion, and low toxicity make it a safe method to use for lymphatic imaging.[8] An imaging system consisting of the excitation light, a near-infrared detector camera, and a high-resolution monitor is necessary for real-time tracing of the superficial lymphatic system. The aim of the study was to investigate changes of the lymphatic system in patients who underwent soft-tissue reconstruction for severe compound fractures, Gustilo type IIIB tibial fracture, by using indocyanine green fluorescence lymphography.

PATIENTS AND METHODS

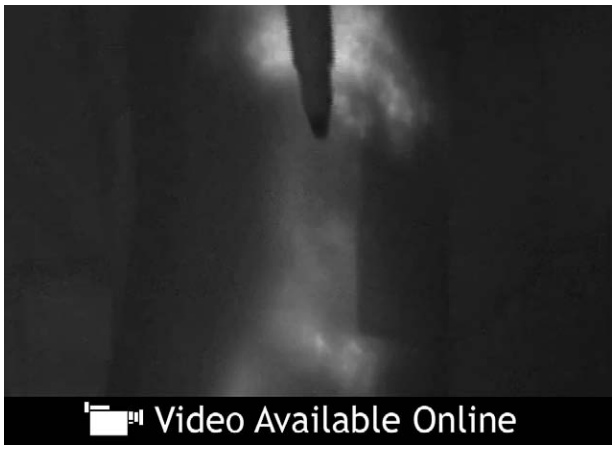
Recruitment commenced after approval from the Royal Adelaide Human Research Ethics Committee. Patients aged older than 18 years who underwent soft-tissue reconstruction for severe compound fractures, Gustilo type IIIB tibial fracture, between January of 2009 and December of 2014 were recruited, and written consent was obtained. Patients were invited for a 1-year follow-up in 2015. Exclusion criteria consisted of the following: not residing in South Australia and inability to attend the clinic, inability or unwillingness to give informed consent, known reaction to indocyanine green or allergy to iodides, pregnancy, pain, or any infection precluding indocyanine green lymphography. Baseline demographics, mechanism of injury, orthopedic and soft-tissue reconstruction, and postoperative complications were recorded.

Indocyanine Green Fluorescence Lymphography

Patients were given two 0.1-ml intradermal indocyanine green (Pulsion Medical, Feldkirchen, Germany) injections in the dorsum of the feet proximal to the toes using an insulin needle (dilution of 25 mg/5 ml water). A custom-made near-infrared camera (Flinders Biomedical Engineering, Flinders University and Flinders Medical Centre, Adelaide, South Australia, Australia) was initially used to image superficial lymphatic vessels. Details of the custom-made near-infrared camera are described in our previous report.[9] Subsequently, a commercially available camera was sourced for further imaging (Photodynamic Eye; Hamamatsu Photonics, Hamamatsu, Japan/SDR Scientific, Sydney, New South Wales, Australia). These systems produced identical imaging of the indocyanine green fluorescence. Digital recording software was used to record real-time uncompressed video and to select still images from the videos.[10] [**See**



Video 1. Supplemental Digital Content 1 demonstrates patient 6 with free gracilis muscle flap reconstruction. From the points of injection (bright fluorescence spots at the first and fourth web spaces), the indocyanine green uptake reaches the edge of the flap, where it shows retrograde flow dispersing to the lateral and medial sides of the foot. Lymphatic vessels and dermal backflow areas are highlighted with a skin marker. Pooling indocyanine green injection distal from the flap is visible from the medial side of the foot on the *bottom right* of the video, ***http://links.lww.com/PRS/C34***.



Video 2. Supplemental Digital Content 2 demonstrates patient 17 with latissimus dorsi free muscle flap reconstruction. From the dorsal foot injection site, the indocyanine green uptake reaches the midtibia, where the proximal flow ceases and shows pooling of lymph in a stardust-like pattern (lateral to the muscle flap). From the stardust pattern, two vessels emerge and continue following the great saphenous vein anatomy, converging with a lymphatic vessel that seems to have followed the lateral side of the muscle flap. Light pressure with manual manipulation shows some movement of the indocyanine green, ***http://links.lww.com/PRS/C35***.

Video, Supplemental Digital Content 1, which demonstrates patient 6 with free gracilis muscle flap reconstruction. From the points of injection (bright fluorescence spots at the first and fourth web spaces), the indocyanine green uptake reaches the edge of the flap, where it shows retrograde flow dispersing to the lateral and medial sides of the foot. Lymphatic vessels and dermal backflow areas are highlighted with a skin marker. Pooling indocyanine green injection distal from the flap is visible from the medial side of the foot on the *bottom right* of the video, ***http://links.lww.com/PRS/C34***. **See Video, Supplemental Digital Content 2**, which demonstrates patient 17 with latissimus dorsi free muscle flap reconstruction. From the dorsal foot injection site, the indocyanine green uptake reaches the midtibia, where the proximal flow ceases and shows pooling of lymph in a stardust-like pattern (lateral to the muscle flap). From the stardust pattern, two vessels emerge and continue following the great saphenous vein anatomy, converging with a lymphatic vessel that seems to have followed the lateral side of the muscle flap. Light pressure with manual manipulation shows some movement of the indocyanine green, ***http://links.lww.com/PRS/C35***.] Both legs were imaged, and the unaffected one served as a control. Recording was in the supine position, and participants were asked to move their ankle and activate the calf muscle to enhance lymphatic activity. Time was recorded and comparison was made with the control leg. Imaging continued for approximately 15 minutes per limb, depending on distance of indocyanine green travel in time. Two separate cases received an additional intradermal injection of the same dilution and quantity within their free muscle flap.

RESULTS

Eighty-four patients who underwent bone and soft-tissue reconstruction recorded as Gustilo type IIIB tibial fractures were invited to participate in the study. Fifty-five patients were excluded from the study (29 could not be contacted, 21 opted out, two moved interstate, and three had active infection). Overall, 29 expressed interest, of which 22 have been able to attend the clinic and complete the study assessment. Five patients were excluded from indocyanine green imaging because of flap location on the heel, flap location on the foot, active hepatitis B infection, neuralgia of the perireconstructed site, and a history of recurrent cellulitis. The included patients had reconstructive operations as outlined in Tables 1 and 2: nine free flaps (eight muscle flaps with split-thickness

Table 1. Free Flap Reconstruction

Patient	Sex	Age (yr)	Mechanism	Follow-Up (mo)	Fixation	Reconstruction	Infection	Flap Coverage (days)	Lymphedema
11	M	28	MVA	62	Plate	LD	Osteomyelitis	18	Yes
2	M	73	MVA	44	Plate*	ALT	No	11	Yes
13	M	59	MBA	44	IMN plate*	LD	Osteomyelitis	8	No
15	M	49	Fall	41	Plate	Gracilis	Osteomyelitis	9	No
6	M	26	MBA	30	Plate*	Gracilis	Osteomyelitis	4	No
16	M	37	Crush	26	Plate*	LD	Osteomyelitis plus soft tissue	22	Yes
14	M	55	MVA	14	Plate	Gracilis	Soft tissue	19	Yes
12	M	71	Fall	10	Plate	Gracilis	Soft tissue	15	No
17	M	28	MBA	2	IMN*	LD	No	8	No

M, male; MVA, motor vehicle accident; MBA, motorbike accident; IMN, intramedullary nail; LD, latissimus dorsi; ALT, anterolateral thigh.
*External fixation.

Table 2. Local Soft-Tissue Reconstruction

Patient	Sex	Age (yr)	Mechanism	Follow-Up (mo)	Fixation	Type of Soft-Tissue Reconstruction	Infection	Flap Coverage (days)	Lymphedema
9	M	44	Fall	52	IMN	Local flap	Osteomyelitis	6	No
3	M	34	Metal cable	39	IMN	Local flap	Osteomyelitis	1	No
4	M	68	Fall	39	IMN	Local flap	Soft tissue	3	No
5	M	33	Crush	39	Plate	Local flap	No	4	No
7	F	38	MBA	31	IMN	FT graft	No	1	No
1	F	70	MVA	36	Plate/IMN*	Local gastrocnemius	No	4	No
8	M	33	MBA	31	Plate*	Local flap	No	8	No
10	M	65	Crush	29	*	Local flap	No	5	No

M, male; F, female; MBA, motorbike accident; MVA, motor vehicle accident; IMN, intramedullary nail; ft, full-thickness.
*External fixation.

skin grafting, and one fasciocutaneous flap), one full-thickness skin graft, six local fasciocutaneous flaps, and one local gastrocnemius rotation flap.

The majority of patients were male [$n = 15$ (88 percent)]. The mean age at presentation was 44 years (range, 26 to 73 years). The mean weight was 83 kg (range, 56 to 106 kg), with a mean body mass index of 31 kg/m^2 (range, 24 to 42 kg/m^2). The mean follow-up time between accident and first study assessment was 36 months (range, 2 to 62 months). Vacuum-assisted closure before reconstruction was used for an average of 5 days (range, 2 to 50 days). Of this study population, the mean hospital stay was 21 days (range, 7 to 97 days), with four patients suffering from soft-tissue infection and six patients suffering osteomyelitis while being inpatients.

Results of Indocyanine Green Fluorescence Lymphography

From the indocyanine green images obtained, all participants showed functional differences between the affected and unaffected legs. On average, indocyanine green fluorescence was observed to be slower from the injection site to cross over the talus in the affected leg compared with this same pathway in the contralateral limb, suggesting a reduction in the transport capacity. Of the participants with free flap reconstruction, six showed a dermal backflow pattern in the perireconstructed area (Figs. 1 and 2). The remaining three free flap participants showed the lymphatic vessels adapting by completely avoiding the reconstructed site (Fig. 3). Interestingly, within all of the free flaps, no functioning lymphatic vessels were noted. The presence of palpable edema in five free flap cases was consistent with the indocyanine green dermal backflow patterns.

All local flaps, including the full-thickness skin graft of one participant, had scar tissue blocking the lymph flow and impairing lymphatic vessel continuation (Fig. 4). The lymphatic vessels that were present appeared torturous in some places, avoiding scar tissue and the reconstructed area completely, especially if they were within the region of the normal lymphatic pathway (Fig. 2). In two cases, lymph flow was seen diverting from the great saphenous vein area toward the posterior side to follow the small saphenous vein. This can be expected when injection occurs near the Achilles tendon; however, in these cases, the injection was on the dorsal foot, making the observed detouring drainage pattern. One case demonstrated retrograde flow toward the sole of the foot, as the scar tissue and flap covered most of the ankle (Fig. 1).

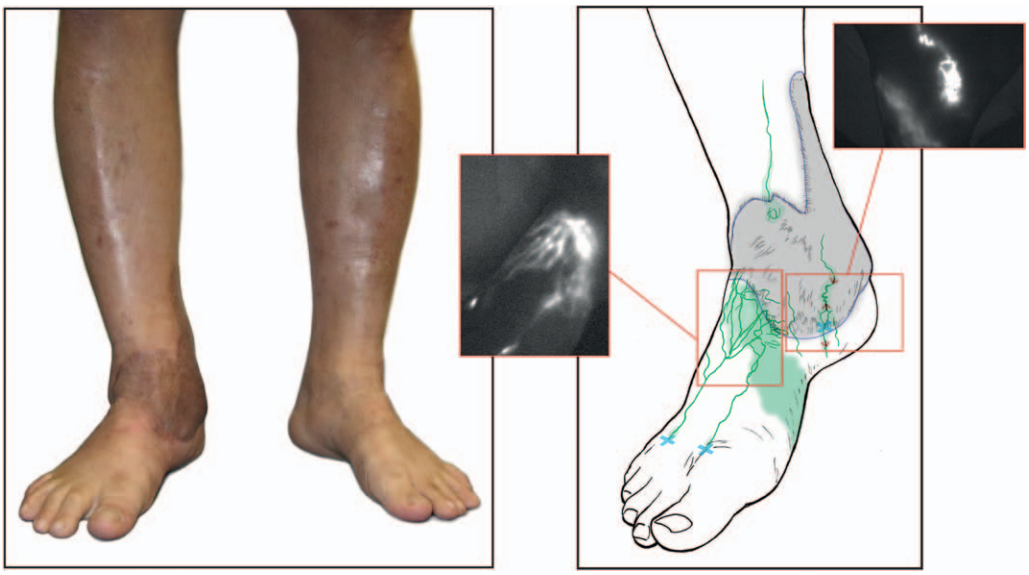


Fig. 1. Patient 6 with free gracilis muscle flap reconstruction. From the point of injection (*X*), the indocyanine green uptake reaches the edge of the flap, where it shows retrograde flow toward the lateral dorsal side. After light pressure on the injection site under the flap, indocyanine green uptake starts ascending and descending at the same time toward the sole of the foot.

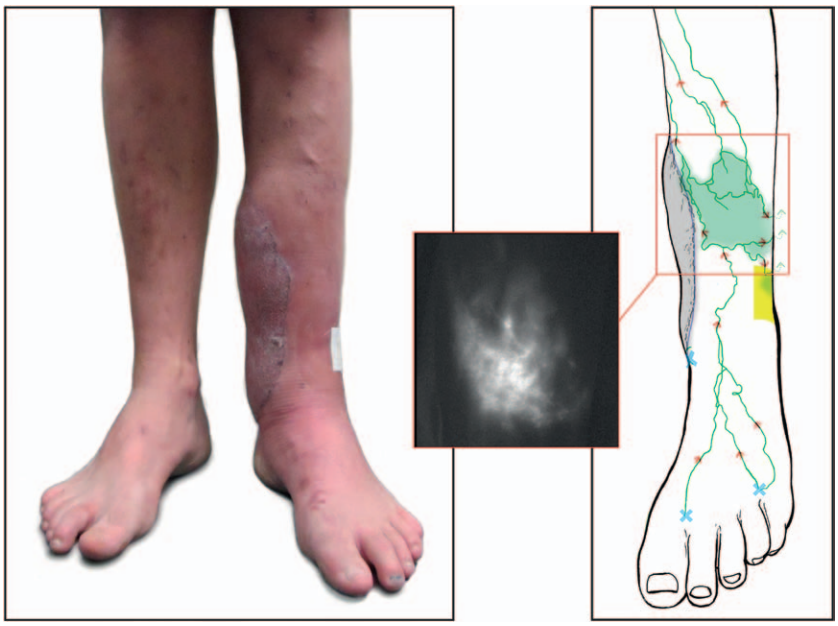


Fig. 2. Patient 17 with latissimus dorsi free muscle flap reconstruction. From the dorsal foot injection site (*X*), the indocyanine green uptake reaches the midtibia, where the proximal flow ceases and shows pooling of lymph in a stardust-like pattern (lateral to the muscle flap). From the stardust pattern, two vessels emerge and continue following the great saphenous vein anatomy converging with a lymphatic vessel that seems to have followed the lateral side of the muscle flap.

DISCUSSION

This study shows the use of indocyanine green fluorescence lymphography imaging in post–lower limb Gustilo type IIIB trauma patients with subsequent soft-tissue reconstruction. Functional lymphatic vessels did not appear in free flaps in the study sample at an average of 36 months after their flap reconstruction. The control limb showed fast uptake of the indocyanine green dye compared with the affected limb. In the local

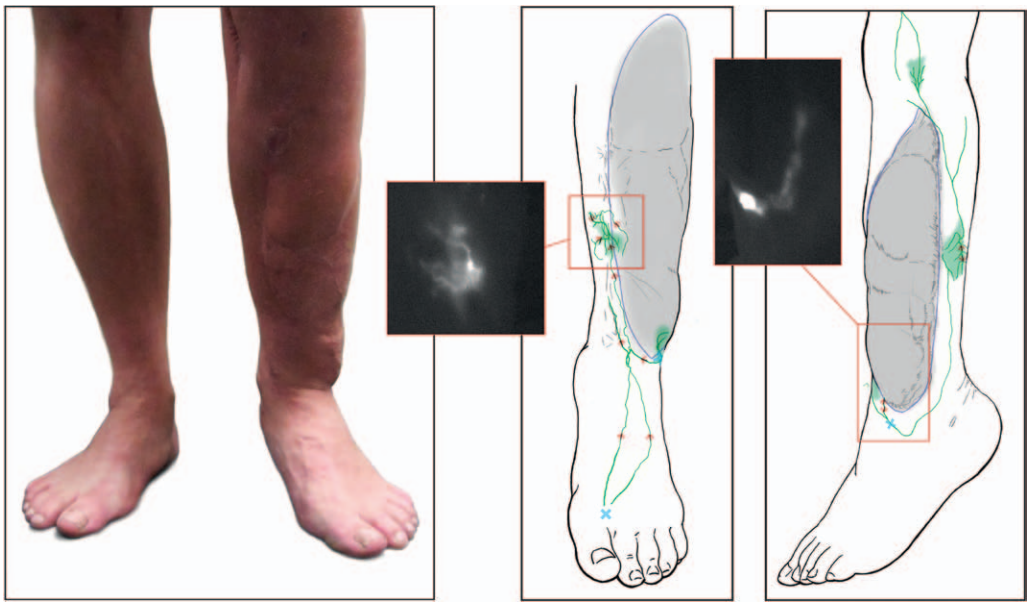


Fig. 3. Patient 16 with latissimus dorsi free muscle flap reconstruction. The point of injection (*X*) on the dorsal foot proximal toes and distal flap shows complete circumvention of the flap. From the distal flap injection site, two lymphatic vessels emerge, with one vessel following the medial line of the tibia and a second vessel merging lateral and eventually following the small saphenous vein. Lymphatic vessels break into a medial vessel following the tibia and merging with the lymphatic vessels ascending from the toe injection. Lateral vessels merge with the small saphenous vein and are blocked by scar tissue in the popliteal region.

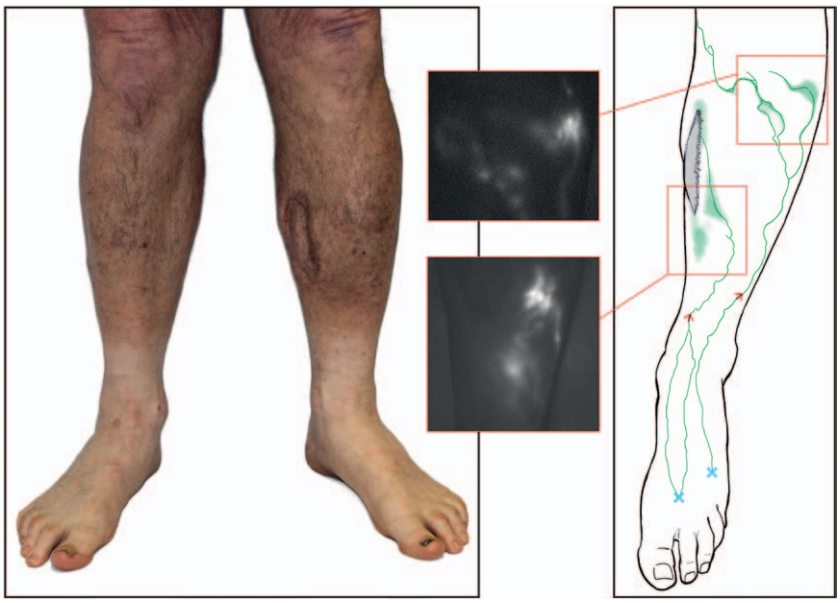


Fig. 4. Patient 10 with local flap reconstruction. The point of injection (*X*) on the dorsal foot proximal toes shows uptake of indocyanine green following the anatomical lymphatic drainage pathway. Patches of dermal backflow are observed distally from the knee and dermal backflow around the flap. No superficial lymphatic activity observed within the reconstructed site.

reconstruction and the free flap reconstruction group, it was evident that scar tissue blocked the continuous flow of superficial lymphatics and seemed to cause local dermal backflow patterns.

Indocyanine green lymphography and subsequent patterns have been described in the literature.[11,12] With the superficial limitation of this imaging technique, only a functional assessment of the present dermal lymphatics can be made. Dermal backflow is one of the first signs of functional failure of the superficial lymphatic vessels, thus increasing the risk for recurrent infections and chronic lymphedema.[1] The limitation of indocyanine green lymphography is penetration depth of the emitted near-infrared light from the indocyanine green compound. We could not find any distinct indocyanine green fluorescence within the free flap, but this finding did not exclude the possibility of any lymphatic vessels existing in the deeper aspect of the free flap.

We did not find any recanalization between the transferred flap and the recipient site in this study. However, we speculate that if the fasciocutaneous flap would be harvested from rich lymphatic vessel areas such as the medial thigh flap, this may facilitate bridging the lymphatic gap. More detailed investigation is required before this theoretical assumption can be clinically implemented.

Lymphatic regeneration through scar tissue, skin grafts, and burn wounds has been observed and reported in both human and animal studies. Research by Amann-Vesti et al. examined the application of split-thickness skin grafts to chronic venous ulcers and the subsequent lymphatic regrowth. They used fluorescence microlymphography 71 months on average after the initial skin grafting. Successful fluorescence microlymphography was performed in all 15 grafts, but only two grafts indicated reestablished viable lymphatic vessels based on the diameter of the vessels and the apparently normal flow.[13] They concluded that functional microvascular lymphatic regrowth remains questionable in meshed skin grafting for venous ulcers. In a different research cohort, the same authors claimed complete lymphatic regrowth within 4 weeks after deep tissue burns. The follow-up at 6 and 18 months suggested completely normal lymphatic functioning. It is likely here that better débridement in burns patient was the likely cause of the improved lymphatic regeneration.[14]

These articles highlight the importance of preparation of the wound bed. This is essential for facilitating normalized tissue homeostasis, creating optimal tissue granulation, and thus establishing the best possible outcome.

With reconstruction using free muscle flaps, vascularized autologous tissue will ensure limb salvage and fracture and soft-tissue healing. Revascularization shown by perfusion will notify the clinician of a successful outcome. The microvasculature within a free muscle flap has the ability to regenerate rapidly. Less information is known regarding the presence of lymphatic vessels within the muscle fiber bundles adjacent to the arteries and veins of the microcirculation. Our clinical study suggests that there were no viable superficial lymphatic vessels demonstrated within the muscle flap reconstruction. This could potentially be attributed to the paucity of lymphatic vessels within the muscle flap. Scarring on the muscle flap and on the edges may prevent the regrowth of lymphatic vessels from the healthy tissue outside of the flap moving into the flap. This concept is supported by earlier work by Suami et al.[11] that reviewed scarring caused by prior graft surgery in a cadaver leg. They explored lymphatic vessel disruption caused by the scar with indistinct vessels between the scar and the surrounding tissue.

Indocyanine green lymphography in local flaps and skin grafts showed a different image. The lymphatic vessel morphology seems to have adapted to go around the reconstructed site compared with their contralateral limb. Despite this being a significant finding, we still have to question our knowledge of the lymphatic anatomy of the individual, because most of our current anatomical information is based on animal studies, human cadavers, or anatomical books with drawings that are more than 100 years old.

Some studies have looked at the anatomy of the superficial lymphatic system, lymphatic recanalization after free flap reconstruction, and specifically at the development of chronic lymphedema.[15] Slavin et al. studied 10 participants (eight lower limbs) with free flaps and investigated the functionality of the large lymphatic collectors. Technetium-99m antimony trisulfide colloid was injected into the flap, and visibility of the tracer and time of uptake were noted. The authors concluded that there was a barrier created by the existing scar tissue but that normal lymphatic functioning seemed to be restored.[5] Perhaps selection of free flap may be different from our findings because their cases were reconstructed by musculocutaneous and fasciocutaneous flaps but ours were muscle flaps with skin grafting. The skin paddle of a musculocutaneous flap should contain healthy lymphatic vessels,

which may facilitate reconnection with the recipient lymphatic vessels.

In another clinical study completed by Szczesny and Olszewski, 21 closed lower limb bone fractures and soft-tissue injuries were investigated using isotope lymphography. It was evident from the images that all participants ($n = 30$) appeared to have a reduced transport capacity because of dilated lymph vessels and that in some patients this was also attributable to the occurrence of enlarged inguinal lymph nodes. This study concluded that posttrauma patients are at high risk for recurrent infection and chronic lymphedema.[16] Lohrmann et al. supported these findings with their lymphography results. They observed torturous lymphatic vessels and lymphedema in patients with extensive lower limb soft-tissue trauma.[17]

Indocyanine green fluorescence lymphography is now increasingly used as a diagnostic tool for chronic lymphedema. Unno et al. explored indocyanine green in patients diagnosed with lymphedema and proposed a quantifying method of an otherwise subjective assessment. In both of their studies, the authors recommended indocyanine green use as a diagnostic tool and highlighted that it is safe to use and less time consuming.[18,19] Yamamoto et al. took indocyanine green patterns in lymphedema patients one step further, where they proposed a direct correlation between lymphedema severity and the indocyanine green pattern. This has been adopted by other researchers; however, opinions are still divided over the diffuse patterns.[12]

More experience with indocyanine green imaging will increase understanding of the different patterns. Regardless of the positive recommendations indocyanine green has received, there is still a consensus to be reached regarding its use for lymphatic imaging, as the depth of imaging remains variable. Indocyanine green lymphography does not penetrate more than 2 cm; therefore, the imaging that occurs is definitely limited to superficial lymphatic vessels.

CONCLUSIONS

Our research has specifically targeted patients with extensive soft-tissue loss and reconstructive surgery. We have successfully demonstrated in this clinical study that lymphatic flow is affected by reconstructive surgery following deep soft-tissue injury. It is also clear that scar tissue is obstructive to normal lymphatic flow and that free flaps seem to have no normally functioning lymphatic vessels.

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