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New Diagnostic Modalities in the Evaluation of Lymphedema

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Abstract

Objective—Currently lymphedema (LED) is typically diagnosed clinically on the basis of a patient's history and characteristic physical findings. While the diagnosis of LED is sometimes confirmed by lymphoscintigraphy (LSG), the technique is limited in its ability to identify pathology and guide therapy. Recent advancements provide opportunities for new imaging techniques to not only assist in the diagnosis of LED, based on anatomical changes, but also to assess contractile function and to guide therapeutic intervention. The purpose of this contribution is to review these imaging techniques.

Methods—Literature for each technique is reviewed and discussed and the evidence for each of these new diagnostic techniques were assessed on several criteria to determine if each could: 1) establish whether disease is present; 2) determine the severity of the disease process; 3) define the pathophysiology of the disease process; 4) demonstrate whether intervention is possible as well as what type; and 5) objectively grade the response to therapy.

Results—LSG is currently the standard test to confirm LED. Duplex ultrasound (DUS) is a simple, readily available non-invasive test that can identify LED by specific tissue characteristics and the response to therapy. MRI and CT scans similarly demonstrate the alterations in epidermal and subcutaneous tissue, but the latter can also detect obstructing neoplasms as a cause of secondary LED. Moreover, MR lymphangiography details lymphatic vessels and nodes and their function. Newer fluorescence imaging techniques provide opportunities to imaging lymphatic anatomy and function. *Visible* micro lymphangiography by fluorescein sodium is limited by tissue light absorption to imaging depths of 200 μ m. Near-infrared fluorescence (NIRF) lymphatic imaging [NIRF-LI], a newer test using intradermal injection of indocyanine green, can penetrate several centimeters of tissue and can visualize the initial and conducting lymphatics, the lymph node basins, as well as the active function of lymphangions (the key module) in exquisite detail.

Conclusions—The availability and the non-invasive nature of DUS should make this modality the first choice for establishing the diagnosis of LED based on tissue changes. Further studies

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comparing DUS to LSG, however, are needed. The costs of MRI and CT limit their adoption as a means to regularly assess the lymphatics. While lymphatic truncal anatomy and transit times can be delineated by the older technique of LSG, NIRF-LI is rapid, highly sensitive, repeatable, and provides exquisite detail for lymphatic vessel anatomy/function of the lymphangions as well as the response to therapy.

INTRODUCTION

The lymphatics have been called the forgotten child in the vasculature family. Despite occurring roughly in 1 out of 30 individuals worldwide, lymphedema (LED) may actually be diagnosed infrequently by physicians overall. This problem can be related to physicians' unfamiliarity with the disease and the lack of readily available, non-invasive diagnostic tests. The 2013 Consensus Document of the International Society of Lymphology (ISL) defined LED as "an external (or internal) manifestation of lymphatic system insufficiency and deranged lymph transport". Over 70 years ago Allen and associates[1] classified LED into: 1) **Primary**-due to in utero developmental defects, which has a prevalence of 1.15 cases/100,000; and 2) **Secondary**-related to acquired causes such as cancer therapy, which, in the US, has been estimated to impact over 11.4 million cancer survivors. Kinmonth further subdivided Primary LED by time of onset into: *Congenital*-present at birth; *Praecox*-the most common of the three, which develops before age 35 often at puberty; and *Tarda*-occurring after age 35.[2] It is the purpose of this review to focus on the diagnosis of lymphedema and how newer techniques can be incorporated into the diagnostic algorithm, guide therapy and assess the results of treatment.

Physiology and pathophysiology of the lymphatics

Our knowledge of lymphatic function has grown over the last decade and an appreciation of this pathophysiology is essential to understanding the basis for these tests, where specific diagnostic tests should be employed and what they may show. Unlike the closed hemovascular system (arteries and veins), the lymphatic vasculature is an open system, which evolved to move macromolecules, immune cells, and excess interstitial fluid (ISF) from tissues, through the lymph nodes for immune surveillance, and ultimately emptying the fluid or lymph into the hemovascular system through the subclavian vein (Figure 1). In contrast to the arterial and venous systems, the lymphatics lack a centralized pumping organ, so that lymph is transported through the lymphatics, primarily by cyclic contractions of vessel subunits called lymphangions. (Figure 1) Entry into the lymphatics occurs in the immature, blind-ending lymphatic capillaries that lack a basement membrane and consist of oak-leaf shaped lymphatic endothelial cells (LECs). These cells are connected to the extracellular matrix through fibrin tethers, which enables the uptake of ISF, immune cells, and particles of diameter <1 μm . These capillaries or "initial lymphatics" are located beneath the epidermis as well as in the tunica adventitia of organs and blood vessels.

Once within the initial capillaries, lymph passively drains into more mature, collecting and conducting vessels which gain a basement membrane and are lined with smooth muscle cells. The conducting vasculature is comprised of "lymphangions" or vessel segments bounded by valves and surrounded by layers of contractile, smooth muscle cell that, through

a concert of intraluminal valve openings, closures, and smooth muscle contractions, pump lymph unidirectionally from one bounded segment to the next. (Figure 1) Lymphangions are also found in the central lymphatic duct (the thoracic duct) and are responsible for the dynamic pressure gradients that are needed to pump lymph proximally (often against gravity in the lower extremities), and to create a reduced pressure or “suction” that draws fluid into the initial lymphatics. While not completely understood, it is thought that membrane depolarization and Ca^{2+} influx is responsible for the lymphatic pump whose frequency and strength may be mediated by pro-inflammatory cytokines, neurotransmitters, sodium and potentially calcium channel blockers.[3–7] When individual lymphangions fail to pump efficiently, lymphatic reflux (akin to venous reflux) and lymphatic congestion can occur. However in the lymphatics, reflux can result in increased “back pressures” causing dermal backflow, or reverse lymph flow into the open initial capillaries and potentially into the extravascular spaces (Figure 1).

Peripheral lymphatic pump dysfunction

Lymphatic reflux has been shown to occur under inflammatory conditions that dilate lymphatic vessels disabling the complete closure of valve “leaflets” and limiting lymphangion pump effectiveness.[7] Furthermore, pro-inflammatory cytokine levels are elevated in both peripheral arterial disease [8] and chronic venous disease.[9] Interestingly, it has been shown from lymphoscintigraphy studies that lymphatic insufficiency in patients with lymphedema-distichiasis may occur in concert with venous reflux as detected by Duplex ultrasound,[10] which is consistent with the common molecular mechanisms of valvular pathogenesis in veins and lymphatics.[11] The net result of combined venous and lymphatic valvular dysfunction are increased capillary filtration due to venous hypertension, reduced lymphatic egress, and concomitant edema that may require clinical attention to both venous and lymphatic vasculatures for effective resolution. Other causes of edema, which are less dependent upon the hemovascular system, may arise from anatomical congenital defects leading to hypoplasia or hyperplasia of lymphatic vessels, as occurs in primary LED. [12] Whether it is lymphatic hypoplasia defects that limit lymph egress (such as Milroy’s disease), or lymphatic hyperplasia that may require greater lymphatic pump pressures to maintain lymph flow across a larger net cross sectional lymphatic vessel area, it is essential to identify therapeutic strategies to move lymph. Beyond showing that LED is present, it is important that a diagnostic test define the defect for therapy to correct. Knowing whether anatomical, functional, or combined anatomical/functional lymphatic deficits may be responsible for fluid retention can help guide effective therapies, which are directed to enhancing lymphatic transport in intact lymphatic vessels using manual therapies, pharmaceutical strategies to combat chronic inflammation (such as biologics to neutralize $\text{TNF-}\alpha$ and other pro-inflammatory cytokines), or surgically restoring lymphatic drainage through lympho-venous anastomoses,[13] lymphatic vessel implant,[14] or autologous transplant.[15] As discussed below, the techniques of lymphoscintigraphy and near-infrared fluorescence (NIRF) imaging can provide information on anatomy as well as function.

Central lymphatic pump dysfunction

Besides being responsible for the uptake of materials into the initial lymphatics and effective transport through the conducting vessels, lymphangions are also responsible for maintaining

lymphatic pressures in the thoracic duct and pumping lymph to the subclavian vein, where, when venous pressure is less than the lymphatic return pressure, lymph is returned to the venous vasculature. (Figure 1) Increased venous pressure in the venous return system or reduced lymphatic fluid pressures in the thoracic duct can prevent lymphatic return to the blood vasculature and can result in impaired lymphatic transport, lymph spillage or leaks resulting in the critical conditions of hydrothorax, chylothorax, as well as chylous ascities. Congenital lymphatic malformations and leaks in the central lymphatics (i.e. the thoracic duct and its tributaries) can also compromise the effectiveness of the lymph pump and are a cause of these potentially fatal conditions. Defining the cause of chylous ascities and chylothorax is essential for proper surgical management,[16] i.e. ligation of a compromised thoracic duct or its tributaries may be indicated when there is a lymphatic “leak.” Microsurgical approaches with direct lympho-venous or reconstructive lymphatic-lymphatic anastomoses show promise.

In summary, while the heart drives blood circulation through the hemovascular system, a network of lymphangions comprise the “pumping” system of the lymphatic vasculature. Knowledge of lymphangion function by a diagnostic study is optimal for disease management. Since the lymphatic system is “open,” anatomical abnormalities can dramatically alter the efficiency of the pump in returning fluid to the blood vasculature, impairing immune surveillance, and thereby creating congestion of immune cells and toxic macromolecules in tissues and blood vessel walls. The chronic accumulation of lymph can cause changes, such as fibrosis, to the surrounding tissues and promote infection. Methods to image both the anatomy and function of the lymphatics provide valuable insights toward mediating the progression of chronic lymphatic conditions, whether through the stimulation of the lymphatic pump through massage or intermittent compression, the neutralization of pro-inflammatory cytokines through immunotherapies, or the surgical correction of anatomical abnormalities.

DIAGNOSIS

Clinical

While LED is usually clinically diagnosed on the basis of history and characteristic physical findings, recent advances in technology provide new opportunities for imaging in the diagnosis and treatment of LED. Herein, we focus on these imaging modalities and their use in lymphatic imaging.

In 2013 The International Society of Lymphology revised the 1995 consensus document on the *Clinical Staging* of lymphedema, similar to those classification systems used with arterial (Rutherford) and venous disease (CEAP-clinical).[17] As shown in Table I, stage zero or 1A is described as a latent or subclinical condition, where limb swelling is not clinically apparent, despite problems with lymph transport. Stage 1 is related to an accumulation of edema fluid high in protein content and this edema reduces with limb elevation. Pitting can also occur during stage 1 disease. Stage 2 is indicated by a lack of reduction in limb swelling with elevation and firmer tissues due to fibrosis. Stage 3 disease represents the most severe form of LED--“lymphostatic elephantiasis”, where pitting is usually absent and marked trophic skin changes are present.

Specific Diagnostic Tests

Historically there has been no simple, non-invasive test accepted for the diagnosis of lymphatic dysfunction, and lymphatic abnormalities are predominately diagnosed by clinical means. This is in stark contrast to the various diagnostic techniques, which are initially non-invasive, that are readily available for the clinical diagnosis of both arterial and venous disease. For example, doppler arterial occlusion pressure, which can be expressed in absolute terms or expressed as the ankle/brachial ratio, objectively demonstrates the presence of arterial occlusive disease and its severity, while Duplex assessment of both acute and chronic venous disease detects acute thrombosis, reflux in the superficial and deep systems, and/or obstruction. A properly designed diagnostic test should: 1) establish whether disease is present; 2) determine the severity of the disease process; 3) define the pathophysiology of the disease process; 4) demonstrate whether intervention is possible as well as what type; and 5) objectively grade the response to therapy. Diagnostic studies are generally divided into: 1) *functional (hemodynamic)*, such as Doppler segmental pressures or pulse volume recordings or 2) *anatomic (imaging)*, such as duplex ultrasound, magnetic resonance angiography, and CT angiography.

The optimum way to determine the diagnostic accuracy of a new test is to compare the agreement of that test to a gold standard (also called the index test) for what is considered to be disease i.e. LED. This comparative study ideally should assess the sensitivity and specificity of the test in a blinded fashion. To achieve the best balance between sensitivity and specificity, the threshold value (that value by which a test is called positive) can be varied in the form of a receiver operating characteristic (ROC) curve.[18] Unfortunately, only a few such studies exist to validate the accuracy of tests to diagnose lymphatic abnormalities.

Overview of diagnostic studies

As summarized in Table II, there are several different techniques to image the anatomy and function of peripheral and central lymphatics. These tests can assess the lymphatics either *directly*, showing the abnormal lymphatic vessels and/or their dysfunction, or *indirectly* by imaging the sequelae of lymphatic dysfunction, such as the development of fibrosis or other abnormal tissue characteristics. Each of these methods are discussed subsequently in this review. .

Lymphography requires an injection of an iodinated contrast agent for radiography or a gadolinium contrast agent for magnetic resonance imaging (MRI). The lymphatic for cannulation and infusion of the agent is identified by injection of blue dye and exposed by a minor surgical procedure. Alternatively, the agent can be injected intranodally under ultrasound guidance.[19] Both methods provide acceptable resolution of anatomical features and are essential for identifying lymphatic malformations and leaks especially in the central lymphatics, as well as being necessary for the diagnosis and treatment of chylous effusions and with thoracic duct embolization.[20] MR and CT can be used to detect contrast-filled lymphatic vessels and are essential to defining the causes of traumatic chylothorax, whether due to "leaks" originating from the central lymphatics thoracic duct, or passage of chylous

ascities into the pleural cavity via retroperitoneal or pulmonary lymphatic malformations or abnormal lymphatic collaterals that originate below the diaphragm.

Lymphography

Scientists have been investigating the lymphatics since the time that the United States was first settled in the 1620's. Like the invention of arteriography and phlebography, it was the development of "lymphangiography", now termed lymphography, both visual and radiologic, by Professor John Kinmonth that greatly advanced our knowledge of LED. Kinmonth perfected the technique of visualizing the wispy lymphatic vessels with an intradermal injection of patent blue dye for visual lymphography, which outlined the superficial lymphatic vessels for cannulation with a specially-designed 30 gauge needle under an operating microscope.[21] Kinmonth then infused an oil-soluble iodinated contrast medium, ultrafluid lipiodol, into the lymphatic vessel. In 1969 Kinmonth proposed a classification of patients with primary LED, which was based on the various anatomic patterns visualized on lymphography in his extensive clinical series of patients with LED at St. Thomas's Hospital, London.[22] The most common pattern was hypoplasia (87%), characterized by a diminished number of lymphatic vessels and nodes, while aplasia, the absence of lymphatic vessels and nodes (5%), and hyperplasia (8%), an increased number of vessels and nodes, were relatively equal in numbers. Kinmonth also described the lymphographic patterns in secondary LED. Following oncologic surgery both the number and diameter of lymphatic vessels are increased to produce a pattern of hyperplasia on lymphography. In the most common world-wide form of LED, which is caused by Filariasis, varicose lymphatics are opacified with numerical hyperplasia, while dynamic cinelymphography showed incompetence of lymphatic valves. These findings are in contrast to secondary LED following recurring bouts of lymphangitis or cellulitis. This process results in fibrosis and narrowing of the lymphatic vessels, which leads to numerical hypoplasia, but the regional nodes are usually hyperplastic.

Owing to the technical difficulty of cannulating lymphatic vessels and the morbidity of the oil based contrast agent, this technique is rarely used today. However, Partsch and colleagues developed a method of indirectly targeting the lymphatic vessels by intradermally administering a water-soluble iodinated contrast agent for uptake into the dermal lymphatics. Using this technique, they were also able to image the lymphatic vessels as well as the backflow of the contrast agent into the dermal lymphatics in a variety of diseases including primary and secondary LED (see Figure 2A)[23]. While this approach does not require direct cannulation of the lymphatic vessels and does not have the same morbidity, it is difficult to deliver sufficient contrast agent to provide contrast across large fields of view and its clinical application has been limited.

Duplex Ultrasound (DUS)

The evaluation of an edematous limb by DUS has usually focused on detecting obstruction or reflux in the venous system in order to demonstrate a venous etiology alone or in combination with a lymphatic abnormality (phlebolymphedema). DUS is a simple, non-invasive and readily available imaging test for the blood vasculature, but while enlarged lymph nodes can be visualized, it cannot image the lymphatic vasculature. The evaluation of

the ultrasound characteristics of the tissue layers in the edematous limb by DUS, however, may provide information on the etiology of the edema, as will be described subsequently. In addition, DUS imaging can grade the severity of the LED, which correlates with the ISL's clinical severity staging, as shown in Figure 2B. Finally, DUS provides an objective measurement of the response to therapy by determining the thickness of each of the tissue elements in the limb before and after treatment.

Suehiro and associates employed DUS to characterize the dermis and subcutaneous tissues of 35 patients with secondary LED.[24] Both the thickness of the skin and subcutaneous tissue, as well as the echogenicity of the subcutaneous tissue correlated well with the ISL clinical staging. Cellular changes, which include hypertrophy of the connective tissue with an increase in the number of fat cells and their hypertrophy as well as the buildup of interstitial fluid, are all demonstrated on DUS. Suehiro and colleagues described the typical DUS image of the tissues as an epidermal entrance echo, which is highly reflective and originates from the outer layers of the epidermis. This pattern is related to "changes in impedance from the coupling medium to the stratum corium". The next layer, the dermis, portrays an array of echoes with various intensities, which is due to ultrasound reflection from the interface between collagen fibers. Finally, the subcutaneous layer "contains horizontal or obliquely oriented echogenic lines caused by connective tissue bundles that represent fat globules and connective tissue septa". Subcutaneous echogenicity can be graded into: 1) none; 2) diffuse with horizontal echogenic lines; and 3) diffuse with increased and blurred echogenic lines. This study demonstrated that both the thickness of the skin and subcutaneous tissue, as well as the echogenicity of the subcutaneous tissue echogenicity grade (SEG) correlated well with the ISL clinical staging with a high sensitivity and specificity. The authors suggested that LED could be ruled in or out by using DUS. (Figure 2B[25])

The same group compared DUS to standard lymphoscintigraphy (LSG), as the "gold standard" for classifying the cause of the edema as lymphatic in 62 patients with leg edema. [26] LED alone was observed in 29/62 (47%) of patients, combined LED and chronic venous insufficiency (CVI) in 8%, and CVI alone in 21%. DUS images of 20 limbs with dependent lower extremity edema and 54 legs with LED were reviewed in a retrospective analysis. The key characteristics were the degree of echogenicity in the subcutaneous tissue and the echo free space (EFS) in a DUS examination of the legs at 8 points. Dependent edema was defined on DUS as: an increase in echogenicity-predominately in the lower portion of the limb with no difference between the medial and lateral sides of the limb, with the EFS most pronounced in the lower leg and in particular on the lateral side. In early LED, however, echogenicity was increased in *both* the medial thigh and the lower leg, while EFS was increased in all parts of the leg. These characteristic DUS findings may help to differentiate these forms of edema.

Lipidema also can be distinguished from LED by DUS. Naouri and colleagues carried out a prospective blinded study of 64 edematous limbs, which employed high resolution DUS (20 MHz).[27] All lower limbs with LED were correctly diagnosed (inter-observer agreement 0.98). Lipidema demonstrated *normal* dermal thickness and echogenicity, while LED

showed the previously described DUS findings of increased dermal thickness and reduced echogenicity.

Lymphoscintigraphy (LSG)

This diagnostic study, which was first described by Sherman in 1953,[28] directly images the lymphatic system by injecting intradermally radionuclides into the interdigital space. LSG has supplanted standard bipedal lymphography, as the currently preferred method for establishing the presence of LED. It is less invasive than lymphography and repeated examinations can be carried out. LSG has been recommended by both the ISL and the AVF Guidelines with a Grade 1 recommendation and B level of evidence from the latter as the initial test for the evaluation of patients with LED. In addition to providing the diagnosis of LED, LSG can not only characterize the severity of LED, and but also assess post-therapeutic results. Due to the use of LSG in sentinel node mapping axillary nodes in breast cancer staging, it is available at most institutions.

Since several types of radionuclides have been used, the specific type will be described with each study. The LSG examination is based on the transport of the radiolabeled substance by the lymphatic system, so that a semi-quantitative measurement of the appearance time of the tracer can be carried out as well as the pattern of distribution of images. Patel et al termed the visual or *qualitative* interpretation of LSG as defining the presence of and caliber of lymphatic vessels, lymph nodes, collateral networks, and delay in radionuclide uptake.[29] By contrast, *quantitative* LSG involves objective measurement of radionuclide transit times, as determined by the uptake and clearance time from the site of injection and clearance time from the limb (Figure 3).

Cambria and colleagues from the Mayo Clinic conducted a prospective study of LSG examinations with technetium 99m-labeled antimony trisulfide colloid in 188 patients (386 extremities) with lower extremity swelling.[30] Following an exercise protocol the patients were imaged, the transport index (TI) or time for transport to regional nodes was employed to measure the disappearance of the tracer. The distribution pattern was also scored. Seventy-nine extremities demonstrated both a normal LS pattern and a mean TI of 2.6 ± 0.5 , while 124 patients with LED demonstrated a prolonged TI (mean 23.8 ± 1.5). There was no difference in the TI between primary or secondary LED. Of interest was that 41 patients had abnormal venous studies, of whom 18 (44%) had abnormal TIs, which is indicative of concomitant LED.

A second large study of LSG by Yuan et al in 110 patients with clinical suspicion of LED demonstrated that the diagnostic findings on LSG for LED were multiple.[31] Using Tc-99m dextran, 82 patients were proven to have LED. The investigators defined a normal pattern on LSG as no residual collection in the soft tissue or lymph channels. By contrast, the differentiating characteristics of limbs with LED on LSG were a lack of visible lymphatic channels and an accumulation of radiotracer in the soft tissue or lymphatic webs. In addition, the pattern seen with primary LED was a high concentration of radionuclide at the injection site and no evidence of radionuclide in the lymphatic pathways or lymph nodes, which is consistent with hypoplasia.

The drawbacks with LSG, however, are that it lacks standardization: on the types of radionuclides employed, their dosage, injection sites, exercise/massage protocols to disperse the radionuclide and the timing of imaging. Because the uptake of the radiocolloid is slow, patients often must wait for minutes to hours after injection before imaging commences. In addition, despite minutes of exposure time the resulting LSG images are grainy with only the lymph nodes and largest lymphatic vessels clearly resolved. Moreover, in patients with early defects in lymphatic function, LSG can be normal with abnormal LSG studies only occurring after extensive dermal backflow and edema is present. Indeed, as shown in Figure 3, Mihara et al. found that fluorescence imaging permitted definitive diagnosis of early and mild LED while LSG did not.[32]

Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) Scans

CT and MRI scans have been employed for the diagnosis of LED, based upon direct and indirect characteristic findings on imaging. As with DUS, the affected skin, subcutaneous tissue layers and the underlying normal muscle are imaged with CT. The subcutaneous tissue exhibits a pathognomonic honeycomb distribution of edema, which is due to fibrotic tissue and fluid surrounding the accumulation of fatty tissue. In addition fluid lakes are apparent, while as in other imaging modalities the skin is thickened. MRI and CT also can display the size and the number of lymph nodes, which is helpful in defining the type of primary lymphedema. CT scans and MRIs are essential in the evaluation of the causes of limb swelling, presumptively due to secondary LED. It has been the one of the author's experience (TFO'D) that several cases per year of LED evaluated by him were due to cancer obstructing the lymphatics, which was detected by CT.

Shin and colleagues retrospectively compared CT findings in 44 patients with edema, 19 of whom had confirmed LED, 11 cellulitis and 14 "generalized edema".[33] Those with LED showed honeycombing and taller than wide appearance of fat lobules, but the former finding (honeycombing) was not specific for LED. In generalized edema bone marrow and subcutaneous edema of the trunk were specific characteristics of that entity ($P < 0.01$). While non-contrast MRI techniques typically cannot detect normal lymphatics or abnormal dynamics, in some cases, as demonstrated by Liu, et al., dilated superficial collectors and deep lymphatic trunks from T2-weighted water signals arising from stagnant lymph or chyle can be detected. In this study, 39 patients with lower extremity LED underwent both LSG and 3D MRI in a comparative study. Although LSG demonstrated enlarged lymphatics and nodes as a "fused band or mass", 3D MR clearly showed dilated superficial lymphatic vessels as well as deep lymphatic trunks in addition to the characteristic tissue changes associated with LED.[34] In a more recent and larger study, with 710 patients with primary or secondary LED, Liu and Zang, administered gadobenate dimeglumine intradermally to obtain contrast-enhanced MR lymphographic imaging. This technique provided detailed anatomic and functional information on both the lymphatic vessels and nodes (see Figure 4), and showed tortuous and dilated collecting lymphatics in greater detail than LS.[35]

Newer Lymphatic Imaging Modalities

Fluorescent contrast agents have also been employed to evaluate lymphatic anatomy and function and are typically injected intradermally to access the initial lymphatics beneath the

epidermis. From the injection site functional conducting lymphatic vessels will carry the agent to regional lymph node basins. Unlike LSG, the technique is rapid, and, in healthy lymphatics, results in immediate uptake and transit of dye. Two dyes have been utilized in humans, fluorescein sodium, which is excited by *visible* light and indocyanine green (ICG), which is excited by *near-infrared* light. Since visible light (300–760 nm) is absorbed by the primary constituents of tissue (blood, water, melanin), fluorescein sodium is useful for micro lymphangiography, which is employed to probe initial lymphatics under the epidermis at depths limited by tissue light absorption to 200 μm . [36] In addition, this agent is employed for identifying lymphatic vessels during lymphatic microsurgeries that have been developed primarily for upper and lower extremity congenital or acquired LE. [37] Surgical microscopes are routinely outfitted for surgical guidance with fluorescein sodium, making this technology widely available for intraoperative procedures. Since near-infrared (NIR) light (780–900 nm) can penetrate 3–4 centimeters beneath tissue surfaces and has little or no confounding autofluorescence, near-infrared fluorescence (NIRF) imaging with ICG has been more recently employed for detecting the initial, conducting, and lymph node basins associated with the peripheral lymphatic system in humans. [38] Due to its potentially high sensitivity, following administration of trace intradermal doses of ICG, images can be gathered quickly on the order of milliseconds, enabling a compilation of “movies” showing the function associated with normal (Figure 5A) and abnormal (reflux) lymphangion activity (Figure 5B)), passive filling of lymphatic vessels that occurs in the absence of lymphangion activity (Figure 5C), as well as normal and abnormal lymphatic anatomy.

Diagnostic Criteria for NIRF—Normal lymphatic anatomy consists of well-defined, linear lymphatics as shown in Figure 5A. Abnormal lymphatic anatomy (Figure 6) is evidenced by tortuous and/or dilated lymphatics, dermal backflow, and extravascular fluorescence in which the dye appears to be within the interstitial space, sometimes with bright clusters of dye. In patients with LED, extravascular dye accumulation, networks of fluorescent lymphatic capillaries, and/or tortuous lymphatic vessels were observed in all symptomatic and some asymptomatic limbs. Statistical models indicated that limbs with LED exhibited significantly lower lymph propagation velocity and contractile frequency of the lymphangions. In diseased lymphatics NIRF lymphatic imaging can show a reduced number of functioning and dilated lymphatic vessels that undergo passive filling with evidence of absent or reduced lymphangion activity. [39] Rasmussen, et al. characterized normal contractile frequencies of 0.4 ± 0.3 propulsions/min in the legs and 1.3 ± 1.2 propulsions/min in the arms of healthy adults while subjects with lymphedema had contractile frequencies of 0.2 ± 0.2 and 0.3 ± 0.3 propulsions/min in their lymphedematous legs and arms respectively. [40] The pumping action of lymphangions creates intermittent periods of suction in the initial lymphatics and oscillatory positive pressures gradients within lymphangion segments, which promotes proximal movement of lymph from the injection sites towards the regional nodal basin. Malfunction of the lymphatic pump can result in slow uptake and/or passive filling of ICG in the initial lymphatics and can create insufficient or even adverse pressure gradients resulting in reflux or backward flow into the initial lymphatics at the site of intradermal injection, at sites with edema, and at sites where self-reported symptoms of “heaviness” are sensed, even though edema may not be clinically evident. It is noteworthy that dermal backflow may occur some distance proximal to the ICG

injection site, nonetheless indicating proximal luminal transport within lymphatics. In advanced Stage II and III lymphedema patients, Unno, et al first visualized “starry-night” patterns wherein upstream ICG drains extravascularly from the initial lymphatics into the interstitial space.[41] Manual lymphatic therapy and/or intermittent pneumatic compression can move this fluid extravascularly towards draining lymph node basins, again potentially preventing fluid stasis and the inflammatory sequelae that result in fibrosis. Finally, in cases of severe fibrosis, initial lymphatic uptake of the ICG may not occur and when it does, results in dermal backflow and extravascular spillage that may not be effectively moved with manual lymphatic drainage or intermittent pneumatic compression.

NIRF imaging has been used in several applications that include: 1) guiding manual lymphatic drainage techniques[42] by allowing therapists to visualize in real-time the functional lymphatic vessels to which manual therapies can be directed, and 2) determining the success of intermittent pneumatic compression in patients with lymphedema or venous stasis ulcers[43]. Interestingly, in the latter study it has been shown that in patients with unilateral venous stasis ulcers, the contralateral limb also has lymphatic reflux and pooling at the medial ankle sites, consistent with venous *and* lymphatic valvular defects. In the earliest stages of NIRF clinical studies, there are several camera systems emerging that are capable of imaging ICG in the lymphatics as well as in the superficial blood vessels in humans. The lack of industry standards in this early, but rapidly evolving imaging modality, however, and the wide variety of device designs have resulted in variable performance.[44]

CONCLUSIONS

Several societies have recommended LSG as the gold standard and confirmatory test for LED. Although guideline recommendations can lag typically behind new developments in the field, changes in guidelines depend on robust evidence, which is needed for DUS. Certainly, the ready availability of and the non-invasive nature of duplex ultrasound could make this modality the first choice for establishing the diagnosis of LED, which is based on tissue changes arising from lymphatic dysfunction. Interpretation of the DUS images by an individual experienced with the characteristics of LED is essential and who is aware of the patient's previous treatment and their response to therapy. This interpretation should be accompanied by the typical clinical findings of LED. Unfortunately, there are few classic properly done studies of this new diagnostic test, which compare DUS to a gold standard, such as LSG, to validate its sensitivity and specificity.¹⁸ Lymphatic truncal anatomy and function can be delineated by MRI lymphography. Finally, NIRF provides exquisite detail not only about the lymphatic vessel anatomy, but also the function of the lymphangions. Table III summarizes the advantages of the available tests for evaluating LED.

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Figure 1.

Schematic of the lymphatic system throughout the body (center). The initial lymphatic capillaries, such as those located beneath the epidermis (lower left), drain to collecting and conducting lymphatics consisting of series of lymphangions, or lymph ‘hearts.’ Lymphangions (upper left) are bounded by endothelial cells and valves and sequentially contract to propel lymph to regional lymph node basins (center, green circles). From the lymph node basins, lymphangions propel lymph through the thoracic duct and its tributaries to ultimately drain into the venous system at the subclavian vein (upper right). When there is an upstream blockage, vessel deterioration, or insufficient lymphatic pump, lymph can flow backwards into the initial lymphatic capillaries and into the extravascular space (lower right).

Figure 2.
(A) Lymphography image showing lymphatic dermal backflow and extravasation of a iodinated contrast agent following subepidermal injection (Reproduced with permission from [23]). (B) Duplex ultrasound images illustrating the tissue changes which occur with lymphatic failure and may be provide diagnostic insight for its diagnosis (Reproduced with permission from [25])

Figure 3.

Example lymphoscintigrams (A, C) and near-infrared fluorescence images (B, D) of the upper limbs of two breast cancer survivors with lymphedema of the right arms. As seen in images (A) and (B) the pooling of contrast agent in the abnormal, right arm lymphatics are clearly visible with both modalities. However, in (C) the lymphoscintigram appears normal while the NIRF image (D) clearly illustrates the abnormal lymphatics in the patient's lymphedematous arm indicating that NIRF imaging may provide opportunities to diagnose earlier stages of LED. Images reproduced from Mihara, et al.[32] under the Creative Commons Attribution license.

Figure 4.

Magnetic resonance images of the lymphatics of a 36 y.o. woman with primary lymphedema of left lower limb, showing (a) numerous tortuous and dilated lymph vessels (arrowheads) in the lymphedematous limb in contrast with contralateral no edema right limb with a few light lymphatic vessels (arrows); (b) enlarged inguinal lymph node with heterogeneous appearance and inhomogeneous enhancement in edema limb (arrows) in contrast with the contralateral nodes that were evenly enhanced (arrowheads); and (c) axial section of T2-weighted MR image shows the thickness and edema of the subcutis (high signal intensity) of the left lower limb. MRL, magnetic resonance lymphangiography. (Reproduced with permission from [33]).

Figure 5.

Near-infrared fluorescence image of lymphatics in (A) the medial right ankle of a normal 57 y.o. male showing linear vessels that actively transport ICG-laden lymph (See VIDEO 1), (B) the medial aspect of the tibial area in a normal 30 y.o. male showing a single dilated lymphatic vessel that actively transports ICG-laden lymph, but shows lymphatic reflux on a dynamic study, and (C) in the dorsal foot of a 47 y.o. male with an infected contralateral foot. While the lymphatic vessels in (C) are demarked by ICG, they lack propulsive motion as shown in a dynamic study. The dark rectangular areas are opaque tape placed over the intradermal sites of injection to prevent camera oversaturation.

Figure 6.

Near-infrared fluorescence imaging in lower extremity LED patients classified with Stage II lymphedema but exhibiting different lymphatic phenotypes of (i) tortuous lymphatic vessels (34 y.o. female), (ii) dermal backflow and extravascular dye (16 y.o. female), (iii) uniform extravascular deposition of dye with no functional vessels (43 y.o. female). Round bandages are placed over the intradermal sites of injection.

Table I

Observed NIRF phenotypes and disease stage.

Disease Stage	(Patho)Physiology	NIRF Phenotype
Normal	Normal physiology	Rapid uptake of ICG; Linear, well-defined lymphatic vessels; Contractile pumping of lymph with reported rates of 0.4 ± 0.3 propulsions/min in legs and 1.3 ± 1.2 propulsions/min in arms
Stage 0 (Preclinical) LE	Initial stages of lymphatic dysfunction; No clinical evidence of LE, although limb may have mild tingling or feel heavy	Linear lymphatics, with possibly dilated or tortuous vessels; Localized dermal backflow may be present in areas that feel heavy; Reduced lymphatic pumping or lymphatic reflux may be present
Stage I LE	Mild swelling that often improves with elevation; May experience pitting edema	Linear lymphatics observed in limb though prevalence is decreased; Localized dermal backflow in edematous areas and occasionally proximal the injection site and edematous areas; Reduced lymphatic pumping; Lymphatic reflux may be present
Stage II LE	Swelling does not respond to elevation; Spongy, non-pitting edema; Skin changes commence (inflammation, hardening, or thickening); Tissue damage irreversible	Few linear lymphatics observed; More extensive dermal backflow and extravascular fluorescence; Reduced lymphatic pumping, when observed, reported rates are 0.2 ± 0.2 propulsions/min in legs, 0.3 ± 0.3 propulsions/min in arms; Lymphatic reflux may be present
Stage III LE	Limbs are large, misshapen, and fibrotic (elephantiasis); Skin is leathery, wrinkled and may have warty protrusions; Infections and fluid leakage common	Few if any observable linear lymphatics; Little or no lymphatic pumping; Extensive extravascular fluorescence and dermal backflow seen throughout limb; In some cases, no uptake of ICG observed

LE = Lymphedema

Table II

Comparison of diagnostic imaging tests for lymphedema.

Test	Contrast Agent and Delivery Method	Focus	Availability/Cost
X-ray Lymphography	Direct vessel cannulation or subcutaneous injection of iodinated contrast agents	Lymphatic vessels and nodes	Variable
Duplex Ultrasound	Endogenous contrast	Tissues Lymph nodes	Readily/Low
Lymphoscintigraphy	Intradermal injection of a radionuclide, such as Technetium-99m	Lymphatic vessels and nodes Contrast Transit times	Readily/High
Magnetic Resonance Imaging/Computed Tomography	Endogenous contrast or various imaging contrast agents	Tissues Lymph nodes	Readily/High
Magnetic Resonance Contrast	Intradermal injection of MR contrast agents such as gadolinium	Lymphatic vessels and nodes Contrast Transit times	Variable/High
Fluorescence micro- lymphangiography	Intradermal injection of fluorescein sodium	Epidermal lymphatics	Variable
NIRF Lymphatic imaging	Intradermal injection of indocyanine green	Initial and collecting lymphatic vessels and nodes Contractile function of lymphangions	Limited/Low to Moderate

Table III

Comparison of imaging modalities and the diagnostic test criteria for lymphedema.

Test	LED Diagnosis	Severity	Pathology	Treatment Possible	Treatment Type	Treatment Response
X-ray Lymphography	Yes	Yes	Yes	No	Yes	No
Duplex Ultrasound	Yes	Yes	No	No	No	Yes
Lymphoscintigraphy	Yes	No	Yes	Yes	Yes	+/-
Magnetic Resonance Imaging/Computed Tomography	Yes	Yes	No	No	No	Yes
Magnetic Resonance Contrast	Yes	Yes	Yes	Yes	Yes	Yes
Fluorescence micro- lymphangiography	Yes	No	No	No	No	No
NIRF lymphatic imaging	Yes	Yes	Yes	Yes	Yes	Yes