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Bridging the divide between pathogenesis and detection in lymphedema

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Abstract

While our understanding of the lymphatic system has improved substantially in the past few decades, the translation of this knowledge into improved healthcare solutions for patients suffering from secondary lymphedema has been severely limited. The challenge facing clinicians is two-fold. First, there is no reliable, affordable, diagnostic capable of detecting the disease before symptoms of the lymphedema develop and the efficacy of treatment options becomes limited. Second, our understanding of the disease pathogenesis, its risk factors, and the underlying physiologic mechanisms is still in its infancy. These two challenges go hand in hand as limited diagnostic options have hindered our ability to understand lymphedema progression, and the lack of known underlying mechanisms involved in the disease prohibits the development of new diagnostic targets. This review serves to discuss the recent developments in clinical and lab research settings of both lymphedema diagnostic technologies and our understanding of the mechanisms driving disease risk and progression. We will show how these two lines of research are synergistically working with the ultimate goal of improving patient outcomes for those suffering from this horrible disease, identifying key areas of further research that are warranted to move the field forward and provide clinical relief for this neglected patient population.

Introduction

Lymphedema is a severely debilitating disease and is often regarded as the most feared survivorship challenge facing patients recovering from breast cancer. While there currently is no permanent cure for lymphedema, early detection of the disease and intervention with compression garments is one of the most effective approaches for limiting its progression (1). The challenge facing clinicians is that what constitutes “early detection” is not grounded in a thorough understanding of the disease pathogenesis, but rather is defined by the limitations of the current state of the art for detection, which routinely is a tape measurer. Of course the lack of availability of techniques and technologies for studying the lymphatic vasculature both clinically and in a research setting has long been an underlying factor in our limited understanding of lymphatic biology in both health and disease. Enhancements in lymphatic diagnostic capabilities (from genomics to proteomics to imaging) directly benefit our understanding of the disease progression, which in turn provide new targets for improving detection technologies and approaches. While current clinical approaches are aimed at capturing the symptoms of the disease (i.e. swelling) as early as possible, recent

research developments suggest that methods capable of detecting underlying deficiencies of lymphatic transport could create a future where we are no longer waiting for the patient's symptom to become severe enough to be detected before we can recommend a course of treatment (Figure 1).

The modern genomic era has given us remarkable insight into the molecular mechanisms involved in lymphatic development (2) and the genetic mutations underlying many types of primary lymphedema (3). Additionally, clinical trials have provided a better understanding of the incidence of the disease for various types of surgical interventions, as well as identified a few broad risk factors that increase one's chances of developing lymphedema (4–6). These topics have been thoroughly reviewed in the papers cited above, and thus will not be a focus of the review here. Rather, this review will summarize both the most recent advancements in the biology of secondary lymphedema progression as well as the development of new diagnostics for early disease detection and evaluation of lymphedema severity. We will show how developments in these areas synergistically work together in the race for a lymphedema cure – 1) better diagnostics are required to successfully detect and correct the underlying dysfunction at its earliest stages; 2) knowing the molecular mechanisms responsible for this dysfunction will provide new diagnostic targets and approaches for further clinical development. Traditional lymphatic diagnostic techniques dating back 50 years or more (e.g. volume measurements, tissue indentation techniques, physical examination) are heavily relied upon for classifying the various clinical stages of lymphedema (Figure 2). Recent developments in lymphedema biology and diagnostic technologies suggest that the progression through these stages could be predicted earlier than classical methods permit. We have chosen to organize this review based upon broad categories of diagnostic techniques that are being explored both clinically and in animal models of lymphedema: 1) methods relying on patient history and routine surveillance of limb volume; 2) methods involving the measurement of lymphatic fluid drainage; 3) methods evaluating the change in the interstitium of the affected area. In each section we will discuss the underlying clinical and biological rationale that motivates the efficacy of the particular methodology as a lymphedema diagnostic, paying attention to the major recent advancements in our understanding of disease progression. This will be followed by an overview of recent developments in the methodologies themselves and how they are contributing to more mechanistic understanding of secondary lymphedema pathogenesis.

Patient History and Routine Surveillance

Rationale

The most widely used diagnostic technique for determining lymphedema remains to carefully track changes in the patient's limb volume. Given that the transition from Stage 0 (or subclinical) lymphedema to Stage 1 is defined as the point at which a detectable change in limb volume occurs, just tracking limb volume alone will never be able to detect subclinical lymphedema (by its very definition) or determine the extent that a person is predisposed. However, recent data has identified several risk factors associated with lymphedema, which could identify patient subsets that can be flagged for more frequent and attentive monitoring. Obesity has been linked with lymphedema risk, development and

severity in numerous clinical trials and animal studies (6–21). While accumulation of adipose tissue is known to be a hallmark of the edematous limb and is seen in nearly all animal models of lymphedema (11, 12, 15, 18, 20), preoperative body-mass-index (BMI) is one of the few predisposing factors for lymphedema that has continuously been demonstrated in clinical trials (6, 13, 14, 16, 17, 19, 21). Utilizing this information coupled with other risk factors that can be easily measured such as elevated blood pressure (17) and the number of lymph nodes removed (6, 8, 22) could provide a powerful tool for identifying those patients most at risk. As genetic screening becomes cheaper and more routinely available, this could provide an additional avenue for risk assessment as several different studies have identified possible combinations of genetic risk factors that could predispose a person to developing secondary lymphedema (19, 23–27).

Diagnosis

One of the most common ways to diagnose and track the progression of lymphedema is through limb volume (LV) measurements, especially in unilateral cases in which the unaffected limb can serve as a control (4, 28, 29). Serial measurement of limb circumference with a tape measure has therefore become the most common LV assessment methodology. This method can be rather time-intensive, requires significant training, and is potentially susceptible to extensive inter-operator variability, but the technique is also comparably inexpensive and has been shown to be reproducible if anatomical landmarks are used to help standardize the procedure (28). Perometry is a 3D LV scanning technique that alleviates some of the operational challenges of the tape measure and water displacement because it is quick and easy to use, mostly automated, and alleviates hygienic concerns. However, it is quite expensive, especially for smaller clinics with lower operating budgets, which has prevented widespread adoption (4, 29, 30). It is also unclear exactly how perometry compares to tape measure assessments; a study by Armer and colleagues found that the tape measure tended to diagnose lymphedema at a higher rate than with Perometry (31).

Given that the rate of lymphedema incidence increases for at least up to 2 years after surgery (8), and early detection is crucial for the best therapeutic outcome, perometry is not an ideal solution as ultimately one needs a diagnostic tool or approach that can be placed in the hands of a patient. This is probably one of the reasons that preemptive surveillance, both in a clinical setting and by the individual patient at home has been one of the more effective approaches (28). Clinical lymphedema surveillance should ideally be performed in regular intervals following a risk-inducing procedure: pre and post-operatively, quarterly for the first year, semi-annually for years 1–3, and annually thereafter (4, 29). However, patients are instructed to constantly monitor the state of their affected extremities for pain, skin changes, increases in size, and feelings of “heaviness” or fatigue (32). Clinical surveillance includes a physical examination of affected limb(s) for signs of soft pitting edema (initial stages of lymphedema) and a focused patient history regarding age, cancer history and associated treatments, family and/or personal history of lymphedema, BMI, post-procedure weight gain, and post-op trauma or infection(s) (28, 33). Patients are also routinely given self-assessment questionnaires, such as the Lymphedema Breast Cancer Questionnaire (LBCQ) or the Gynecologic Cancer Lymphedema Questionnaire (GCLQ), which have been validated as reliable lymphedema diagnostic aids (4, 34, 35).

While, as discussed in the following sections, there have been significant advances in the sophistication of various techniques to measure and quantify lymphedema development at various stages of the disease, the approaches that appear to have the most widespread impact on lymphedema diagnosis to date involve the development of better educational pamphlets and relying on the patient to self-diagnose. This situation is certainly reflective of the value of better patient education, but it also provides insight into the significant gap that remains between cutting-edge lymphatic technologies and the ability to improve patient prognosis for those faced with a life of lymphedema risk.

Failure of lymphatic drainage

Rationale

Given that lymphedema presumably develops due to the inability of the remaining intact lymphatic vasculature to return fluid from the interstitium back to the circulation at the same rate at which lymph is formed, detecting this deficiency would be one of the most promising ways to transition from diagnostic approaches that rely on detecting symptoms of the disease, to those that can detect its underlying cause. Given that lymphedema can present itself several years after surgery, it is possible that the remodeled lymphatic vasculature after surgery is predisposed to failure. This phenomenon was recently demonstrated in a rat foreleg model of lymphedema, in which the lymphatic system was allowed to regenerate for 73 days after an axillary lymph node dissection (ALND) surgery (36). At this time point the animals exhibited no external signs of swelling. However, upon challenge with an inflammatory stimulus, ALND animals showed significant signs of swelling and skin remodeling compared to controls, suggesting that the remodeled lymphatics possessed an underlying deficiency in their transport capabilities that was only detectable when challenged.

Others have provided evidence in vitro, in animal models, or in human lymphedema patients that ineffective lymphangiogenesis could be a driving factor in secondary lymphedema (10, 18, 22, 37–45). It is less clear, however, whether the abnormal lymphangiogenesis, usually described by enhanced proliferation of initial lymphatic capillaries (18, 38–41), the presence of tortuous lymphatic vessels (22, 43, 44), and hyperplastic lymphatic vessels (18), is an underlying cause in the development of the lymphedema, or merely a remodeling response to the highly inflammatory environment of later stage lymphedema. The latter is supported by the fact that these vessel phenotypes are usually noticed in situations where lymphedema has already developed (39), and inflammation itself is known to significantly upregulate lymphangiogenesis (46).

While there are numerous theoretical possibilities in which lymphatic transport might fail, only limited experimental data exists demonstrating correlation (much less causation) between failure of the lymphatic pump and lymphedema development. Collecting vessels have been demonstrated to remodel and become stenotic, with the severity of the stenosis correlating with lymphedema stage (Fig. 3) (47). In a clinical study, Stanton and colleagues demonstrated that breast cancer related lymphedema developed in the arms of individuals with higher peripheral lymph flows, suggesting the higher filtration rates are a risk factor for lymphedema, perhaps by placing demands on the lymphatics that exceed their capacity (48).

Alternatively, upon radiation in a rabbit model it has been shown that the lymph node remodels and increases its resistance to flow, possibly impeding transport of lymph from the downstream afferent vessel (37). Lastly, under modest mechanical loading, the valves of isolated lymphatic vessel chains have been demonstrated to “lock” resulting in an inability to pump flow against an adverse pressure gradient (49). Given that the behavior of lymphatic valves are highly sensitive to pressure (50) and mutations affecting the proper formation of lymphatic valves is one of the most common causes of primary lymphedema (3, 51, 52), it is possible that certain patients could have undetectable valve defects in their lymphatics that fail when faced with the elevated fluid demand placed on them during the earliest stages of lymphedema. Lymphatic pumping is highly sensitive to the mechanical loading placed upon the vessel (53–58) and since lymphedema is usually a battle against gravity, understanding both how the mechanical environment changes during the disease and the consequence of these changes to pump function is an important area of future research.

Diagnosis

The recent evidence regarding the role of lymphatic pump failure in the etiology of lymphedema, has provided renewed promise that a diagnostic approach capable of capturing this failure (or propensity to fail) in the earliest stages would be a significant advancement in patient care. Numerous imaging approaches have been investigated, each with a variety of advantages and disadvantages in terms of cost, accessibility, and ability to capture the various underlying lymphatic defects postulated above. Unfortunately, to date, most of these techniques have been unable to capture subclinical defects. Yet all of them are not-surprisingly, quite adept at demonstrating lymphatic dysfunction in Stage 2 and 3 lymphedema patients, which provides hope that continued advances can provide a subclinical diagnosis.

Lymphoscintigraphy—When imaging is used to diagnose lymphedema, lymphoscintigraphy is still widely considered the gold standard (4). The technique begins with an intradermal or subcutaneous injection of one of a variety of nuclear particles and then relies upon subsequent imaging of the clearance of the particles to provide information regarding the patient’s lymphatic transport function. Lymphoscintigraphy can be used in the diagnosis of lymphedema by examining differences in radioactivity between affected and unaffected regions, calculating clearance rates of radionuclide injections, and by observing dermal backflow of radionuclides in lymphatic vessels. Szuba and colleagues demonstrated the ability of lymphoscintigraphy to quantitatively estimate the radioactivity ratio between the axilla of a healthy arm and an affected arm as well as identify dermal backflow patterns in lymphedema patients (59, 60). A numerical index was also developed specifically for post-mastectomy lymphedema patients in order to assess lymphedema progression based upon observations of dermal backflow and visualization of proximal lymph nodes.

MRI—MR lymphangiography is the second traditional gold-standard in lymphatic imaging. The technique involves the injection (interstitial or intravenous) of various contrast agents and analyzes the alignment, behavior, and interaction of protons when a magnetic field is applied. MR as it relates to lymphatic imaging is most commonly used for visualization of

lymphatic vessels with the advantage of better spatial resolution than nuclear techniques. For example, Lohrmann et al. used MR lymphangiography to visualize lymphatic vessels with dermal backflow in lymphedema patients (61, 62). More recently, MR lymphangiography was used to show that particular lymphatic drainage patterns and other imaging metrics such as “time to enhancement” correlate with lymphedema disease stage in lower extremity lymphedema patients (63).

Others have taken a different approach by exploring unenhanced MR lymphatic imaging. Lu et al. and Matsushima et al. have developed techniques involving heavily T2-weighted MRI (64, 65), and comparing MR lymphangiography with heavily T2-weighted MRI, Lu et al. found that while MR lymphangiography produces better image quality, heavily T2-weighted MRI has a greater sensitivity (66). Alternatively, work by Rane and colleagues has demonstrated the ability to detect differences in lymphatic flow velocity in normal and lymphedematous arms using spin labeling MRI (67), which is an MR technique that does not require exogenous agents for imaging. In the near-term, a combination of MR lymphangiography and noncontrast-enhanced MRI may provide the best approach for lymphedema diagnosis (66), but continued advances towards imaging the lymphatics without contrast agents would be highly valuable.

NIR—An emerging technology that is becoming very popular in the literature is near-infrared (NIR) fluorescence lymphatic imaging. The technique is most commonly performed with the FDA-approved fluorophore indocyanine green (ICG) by injecting the dye intradermally, exciting it with a laser diode, and imaging it with an NIR-sensitive detector as it is taken up by the lymphatic vasculature (68). ICG-based NIR imaging specifically designed for lymphatics was first reported in two separate studies by Sharma et al. and Unno et al. in 2007 (43, 69). The study by Sharma and colleagues showed, for the first time, dynamic propulsive lymph flow in anesthetized swine. The rhythmic contractions of sequential lymphangions and the series of one-way valves force the fluid to flow in discrete “packets” along the lymphatic vessels, a phenomenon that was not previously understood. The study by Unno and coworkers used NIR lymphatic imaging to identify four characteristics of lymphatic flow associated with lymphedema including dermal backflow, extended fluorescence in the injection site, dilated lymph channels, and fluorescence diffusion otherwise known as leaky vessels.

The effectiveness of NIR lymphatic imaging has recently been demonstrated in several applications related to lymphedema. Mihara and colleagues compared lymphoscintigraphy, MRI, CT and NIR lymphatic imaging for the purposes of early diagnosis of lymphedema in the upper limbs and found NIR imaging to be the most advantageous imaging modality based upon the combination of temporal and spatial resolution of lymphatic fluid transport (45). In 2010, Maus et al. used NIR imaging to direct manual lymph drainage (MLD) and subsequently assess the efficacy of the treatment in patients with head and neck lymphedema. Similarly, Aldrich and colleagues showed that the appearance of aberrant lymphatic networks increased with the duration of lymphedema (70). NIR lymphatic imaging has also been shown to be very effective for early-stage diagnosis of lower extremity lymphedema in gynecologic cancer treatment patients by detecting lymphatic backflow and assessing regional drainage patterns (71). Given the complexities of lymphatic

drainage networks, we have performed research to generate quantitative metrics of lymphatic function by analyzing multiple collecting vessels simultaneously (72), and further research in this regard will help to advance NIR lymphatic imaging as an early-stage lymphedema diagnostic.

Pumping Pressure—One of the major advantages of NIR lymphatic imaging over other imaging modalities is the emergence of new quantitative measures of lymphatic pump function, which will be enormously valuable in objectively defining lymphatic health and assessing the progression of lymphatic disease. Unno et al. (73) have developed a novel technique to quickly and precisely measure lymphatic pumping pressure by converting a lymphoscintigraphy procedure (74) to NIR imaging. ICG was injected into the dorsum of the foot of human volunteers and real-time fluorescence images of lymphatic transport were recorded while a custom sphygmomanometer was used to increase the pressure around the legs until lymphatic flow was observed to have stopped. The pressure was then slowly released until lymphatic flow was restored, which was defined as the lymphatic pumping pressure, P_{pump} . Using NIR imaging of ICG transport through dermal lymphatic vessels during this procedure gives a very accurate measure of the exact time and corresponding applied pressure at which lymphatic flow is stopped. Healthy patients were reported to have a P_{pump} of 29.3 mmHg while lymphedema patients had a significantly lower P_{pump} of 13.2 mmHg, indicating reduced lymph transport capabilities in lymphedema patients. In a follow-up study, Unno and colleagues found that lymphatic pumping pressure decreases with age and decreases more rapidly in women of postmenopausal age than any other age/gender group (75), which may have implications for the development and onset of lymphedema. Recent research directed towards improving this technique has focused on automated control to improve measurement precision and the development of additional metrics and procedures to obtain more data regarding lymphatic pump function (76). Given the easy and repeatable operation, precise measurements, and potential for relatively inexpensive hardware, NIR lymphatic pumping pressure may have the highest likelihood of becoming the first subclinical diagnostic of lymphatic pump function.

Interstitial changes

Rationale

The ultimate pathology of the disease clinically presents itself through the gross remodeling of the interstitium and the skin. Thus, it is not surprising that many of the recent discoveries of the biological mechanisms underlying disease development occur largely in the interstitium (77). Lymph formation itself is driven by the balance of Starling forces, and therefore, changes in the oncotic and hydrostatic pressure in the interstitium will alter the demand placed on the lymphatics (78). This fluid must first traverse the interstitium as interstitial flow where it drains into the initial lymphatics. Interestingly both interstitial flow (39, 42, 79–82) and pressure (83) are important cues for initiating and directing lymphangiogenesis. As the physical properties of the interstitium change during the course of the disease, these changes will in turn alter the hydraulic conductivity of the tissue and thus, both the clearance of fluid and the lymphangiogenic response (15, 84). In fact it is the

mechanical changes of the tissue itself (i.e. tissue fibrosis) that is a hallmark of disease severity and its progression to irreversible, Stage II lymphedema (10, 85–88).

While these mechanical changes are significant manifestations of the disease, they are not merely the result of the physical loss of lymphatic transport and interstitial protein accumulation (89). Recent data from numerous animal and human studies of lymphedema have made it abundantly clear that inflammation is a key, if not the most important, factor in driving lymphedema progression, (7, 10, 36, 86, 90–93) particularly as it relates to the changes that occur in the interstitium. Of course inflammation is also helpful in the lymphangiogenic healing response after surgery and if balanced could be beneficial to lymphedema resolution (38, 46, 94). The molecular mechanisms that regulate the switch from a beneficial inflammatory response to chronic, unresolved inflammation in lymphedema (and other lymphatic-implicated disease) is an active and important area of research. Given that inflammatory cytokines have also been demonstrated to affect collecting lymphatic pump function (95–98), it is likely that the consequences of inflammatory driven lymphedema progression not only alter the interstitium, but severely compromise pump function and drainage by the collectors, providing a positive feedback loop that when triggered, leads to the drastic pathologies observed in Stage II and III patients.

Diagnosis

Just as detectable volume changes in the limb have classically been the defining factor used to draw the diagnostic line between Stage 0 and Stage I lymphedema, permanent changes in the tissue itself define the transition from Stage I and Stage II. Therefore, assessment of tissue composition is another method used to diagnose and track the progression of lymphedema. Where these methods differ, lies in their sensitivity to detect tissue changes before permanent remodeling has set in. Again, just like in volume measurements, the methods are focused on detecting the symptoms of the disease as early as possible, rather than the underlying cause.

Perhaps the most common composition assessment is bioelectrical impedance spectroscopy, which measures extracellular fluid content by passing a small electrical current through the tissue. It has been reported that bioimpedance measurements can detect subtle changes in tissue properties indicative of lymphedema onset months before detection is possible with a tape measure, presumably by measuring the early-stages of inflammation and fluid stagnation (99). Variations of the technique have been reported in the literature for more than 2 decades, but recent research in this area has been focused towards enhancing the diagnostic capabilities of the technique, especially for breast cancer survivors. It has been demonstrated that using frequencies below 30kHz is optimal for the assessment of extracellular fluid volume, and such frequencies should be implemented in impedance spectroscopy devices moving forward (100). Several studies by Ward and colleagues have established impedance ratio ranges for lymphedema diagnosis (99, 101, 102), while Kilbreath et al. demonstrated that comparing the postoperative change in extracellular fluid volume of the ipsilateral extremity to a preoperative baseline may provide a more effective diagnosis of edema than comparison to reported thresholds (103). Newman and colleagues

demonstrated that bioimpedance spectroscopy has sufficient sensitivity to assess not only extracellular fluid volume, but also lean mass and fat mass between affected and unaffected extremities of Stage II unilateral breast cancer-related lymphedema patients, which could provide additional diagnostically-relevant metrics to monitor the progression of later-stage lymphedema (104). Most recently, and arguably most excitingly, Ridner et al. recently developed and reported the first self-measurement protocol for bioimpedance testing, which may pave the way for a home-use diagnostic in the future (105).

Tissue composition can also be assessed through various imaging techniques as well, although they have seen relatively limited clinical implementation to date. Dual energy X-ray absorptiometry has been shown to be equally sensitive as bioelectrical impedance spectroscopy for detecting extracellular fluid and lean and fat mass (104). Ultrasound has been used to measure and track changes in soft tissue thickness (106), with Lee et al. able to detect reductions in soft tissue thickness in breast cancer-related lymphedema patients after 2 weeks of complex decongestive physiotherapy. MRI has also recently been shown useful to assess circumferential and volumetric measurements as well as track morphological changes in breast cancer-related lymphedema patients (107).

Tissue texture measurements may also be useful in the diagnosis and tracking of lymphedema because it is well known that as lymphedema progresses, the tissue becomes resistant to compression. Moseley et al. used tonometry, a measure of tissue compressibility, to quantify differences between normal and lymphedematous breast tissue (108). Similarly, Adriaenssens et al. used elastography, a measure of tissue elasticity, to also show a difference between healthy and affected breast tissue (109). However, Suehiro and colleagues were unable to detect differences between normal and Stage II lymphedematous legs (110), which suggests these techniques, in their current state of development, may only be useful in very advanced stages of lymphedema after significant fibrosis and adipose deposition has occurred.

Biomarkers for lymphedema

There is an exciting new idea in lymphatic diagnostics that, when compared to the above-mentioned techniques, really cannot be lumped into the traditional categories of diagnosis. While all of the techniques discussed previously seek to detect and/or predict the anatomical location of lymphedema (e.g. change of tissue properties, impairment of draining lymph vessel, volume change, etc.), Lin and colleagues sought to determine if patients with lymphedema might present with a panel of circulating biomarkers that would distinguish these patients from control through a simple blood test (10). The advantage of such an approach is that it does not require knowledge of the underlying cause or location of presentation of the disease. To demonstrate the proof of principle, skin biopsies were taken from a variety of lymphedema patients with numerous underlying causes. PCR was performed and differentially expressed genes that coded for secreted proteins were analyzed to identify potential protein targets in the serum. The study resulted in a panel of 6 protein biomarkers that successfully distinguished disease from healthy controls with a high degree of accuracy. Interestingly the proteins uncovered are known to be involved in lymphangiogenesis, inflammation, fibrosis, and adipocytokine signaling, all of which have

been recently identified as important underlying mechanisms involved in disease progression as discussed above. Certainly a diagnostic that can be achieved through a routine blood draw, and encompasses many of the underlying biological mechanisms understood to be promising in lymphedema pathogenesis, would be an ideal clinical tool. However, it remains to be seen whether this or a similar approach would be able to detect early or latent disease as all of the patients in this study already had clinical manifestations of lymphedema.

Conclusions

It is difficult to predict which techniques have the greatest potential for diagnostic success since it remains unknown what a disease-relevant change in lymphatic function that is subclinical actually entails. However, as researchers strive to improve upon the capabilities of these approaches to hit a currently unknown target, the diagnostics themselves will provide deeper insight into the mechanisms driving lymphatic failure. As clinicians, basic scientists, and engineers continue to work together to improve technologies, which fosters new mechanistic understanding and drives further technological development, it is hopeful that a brighter future is in store for those suffering from this all-too-often neglected, debilitating disease.

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Figure 1. Relationship between lymphedema diagnostics and disease pathology

Most clinical lymphatic diagnostics have relied on detecting changes in limb volume as early as possible during the disease progression (typically mid Stage 1). However as new diagnostics develop and our understanding of the disease process progresses, there is potential for diagnostics approached to detect various aspects of lymphedema earlier in the development of the disease and perhaps even being able to detect individuals predisposed to lymphedema. This figure summarizes the relationship between the diagnostic and the underlying pathology it is detecting (i.e. the colored circles with each diagnostic correspond to the detected pathology). Solid colors indicate strong support within the literature for the use of the diagnostic or presence of the pathology at the various stages of lymphedema. Dashed lines represent, in these authors' opinion, the potential for each pathology to show up and be detectable by a corresponding diagnostic approach earlier in the disease process.

Figure 2. Stages of lymphedema progression

Stage 0 or subclinical lymphedema marks the period after the initial insult in which lymphatic fluid transport has begun to deteriorate, but clinical manifestations are not yet apparent. The beginning of the swelling cascade marks the shift to Stage I. At this point the limb becomes swollen, may feel heavy, and exhibits pitting edema. Limb volume diagnosis of lymphedema is often performed during the latter part of Stage I, and the disease is generally still reversible at this stage. The shift to Stage II lymphedema is characterized by advanced swelling and the beginning of fibrosis and adipose deposition. The limb begins to feel hard and exhibits non-pitting edema. Limb volume measurements are still performed on Stage II patients, but the disease quickly becomes irreversible as more tissue remodeling occurs. Stage III lymphedema, although rarely seen in breast cancer survivors, is the most advanced stage of swelling and is also called lymphostatic elephantiasis. – Images courtesy of Dr. Charles McGarvey and Guenter Klose.

Figure 3. Staging of lymphedema and the macroscopic anatomical findings in the collecting lymphatic vessels associated with the stages

In the normal type, microvascular networks were found to nourish the largely developed walls of the collecting lymphatic vessels. The microvascular networks were gradually lost with the progression of the disease stages. In addition, the lymphatic vessel lumen was found to be dilated in the ectasia type, which was associated with an increase in endolymphatic pressure. Because increases in smooth muscle cells and collagen fibers are the major causes of the cloudiness and thickening of the lymphatic vessel wall, they were found to be prominent in the contraction type and the sclerosis type. LE: lymphedema. – Reproduced from the work of Mihara et al. 2012 (47).