

Review

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Diagnostic workup of lymphedema

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Abstract

Lymphedema is a chronic and progressive pathological state of tissue swelling caused by congenital or acquired lymphatic abnormality. History, physical and laboratory examinations could help to diagnosis > 90% lymphedema patients. Early stage lymphedema could be challenging to diagnose. The aim of this review is to provide an objective appraisal of current diagnostic methods, such as lymphoscintigraphy, lympho-fluoroscopies, lymphangiography and *etc.* focusing on their respective advantages and weaknesses, and hopefully shed some lights on developing a practical diagnosis modality beneficial to early detection and clinical decision making of lymphedema.

Keywords: Lymphedema, diagnosis, lymphoscintigraphy, magnetic resonance lymphangiography, indocyanine green, tissue dielectric constant, bioelectrical impedance spectroscopy

INTRODUCTION

Lymphedema is a pathological state of tissue swelling due to excess protein-rich fluid accumulation in the interstitial space. The equilibrium between load of lymph fluid and transport capacity of the lymphatics is almost invariably disturbed by either congenital dysplasia of the lymphatic system (primary lymphedema) or acquired impairment of the lymphatic drainage (secondary lymphedema). Contrary to all expectations, lymphedema has been reported to affect approximately 300 million people worldwide. The incidence of primary lymphedema is 1 in 100,000 individuals with that of secondary one being 1 in 1000 individuals^[1]. Its global impact may even be severely underestimated resulting from various diagnosis methods and common neglect of the disease. As a chronic and progressive condition, lymphedema, if left



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untreated, could give rise to disabling physical and psychosocial complications in the long run. Currently, the attention on lymphedema is far from enough resulting in delayed initial evaluation and treatment and poor prognosis. There is no existing cure for lymphedema and current therapies mainly focus on limiting progression and preventing severe complications. Early intervention is proved to be the root of improved prognosis thus highlighting the significance of early detection. Various new and effective diagnostic methods emerge over the years but there are still no standard guidelines for lymphedema diagnosis, let alone early detection. The aim of this review is to provide an objective appraisal of current diagnostic methods, focusing on their respective advantages and weaknesses, and hopefully shed some lights on developing a practical diagnosis modality beneficial to early detection and clinical decision making of lymphedema.

HISTORY AND MANIFESTATIONS

For suspected lymphedema patients, history and manifestations are invaluable and indispensable. The onset of swelling could be diagnostic for lymphedema. Extremity swelling present for less than 3 months or forms soon after lymphatic injury is not consistent with lymphedema. It's very common to see pediatrics-onset in primary lymphedema, boys' present in infancy and girls' during adolescence^[2]. For secondary lymphedema, travels to parasite-endemic area (filariasis), obesity (BMI > 50)^[2], radical cancer treatment for breast, gynaecological, head and neck cancer (nodes dissection, chemotherapy and radiotherapy), nodes biopsy can be crucial risk factors, while family history is more frequently seen in primary lymphedema. Docetaxel-based chemotherapy has been shown to increase the incidence of breast cancer treatment related lymphedema^[3].

Complaints of extremity heaviness and fatigue could be the main manifestation of early stage lymphedema. As it progresses, visible limb swelling and enlargement of circumference take place. Different tools are utilized to assess extremity volume/circumference. Tape measurement is applying a flexible and non-stretch tape to assess the girth of edematous limb at certain points following different protocols. Absolute values are usually converted into volumes using respective mathematical formulae visualizing the limb as a series of truncated cones, cylinders and trapezoidal solids^[4]. Absolute excess volume (affected limb-unaffected limb), excess volume in percent [(affected limb-unaffected limb)/unaffected limb × 100], relative value in percent (affected limb/unaffected limb × 100) and affected leg volume divided by BMI are useful indices in unilateral lymphedema diagnosis. Girth assessment is the most fundamental and commonly used method for its feasibility and economical advantages but is limited by its high inter- and intra-observer variability and poor reproducibility. In water plethysmography, the amount of water displaced after immersing the limb of interest into a water tank equals the extremity volume. It's considered as the criterion standard for lymphedema diagnosis but also deemed impractical in clinical setting for its cumbersome set-up, patient-unfriendly measurement protocol and extra contraindications concerning water. Extremity volume difference > 10%, volume change > 200 mL or circumference change > 2 cm at one certain point are deemed diagnostic, though there are still no standardized cut-off points among health practioners^[5].

A square frame emitting infrared lights is used in perometry. As the frame moves along the limb, information of the interrupted lights is converted into coordinates to reconstruct a 3D model and automatically calculate the volume^[6]. Similarly, three-dimensional imaging systems such as the VECTRA XT surface photo imaging system (Canfield Imaging Systems, Fairfield, NJ) are developed to capture 360° digital image data of the edematous extremity. Absolute values or image color change by photographs contrast presents volume changes before and after treatment, thus making VECTRA valuable in both diagnosis and monitoring^[7]. VECTRA, as a relatively new technique provides high resolution images and might be applied to the whole body, facial and pubic region included^[7,8]. However, both three-dimensional photography and perometry are costly and not obtainable in every clinic.

As edema persists, difficulty of fitting clothing, joint dysfunction and musculoskeletal agony may appear. Characteristic skin changes including peau d'orange (pitted or dimpled skin texture), Kaposi-Stemmer sign (the inability to pinch the fold of skin at the base of the second toe) and squared off appearance of toes assists to identify lymphedema. Hyperkeratosis and fibrosis with verruca and nodules usually indicate advanced stages. Lymphedematous extremity is prone to recurrent infection, cellulitis lymphangitis, lymphorrhea and skin ulceration. Angiosarcoma that initially presents itself as red-purple nodules with/without satellite lesions is a rare but lethal complication.

Laboratory examinations such as routine blood test, thyroid function or urinalysis are in need to rule out other causes of edema, including renal, heart or hepatic failure *etc.* Though thorough history, physical and laboratory examinations could help to diagnosis > 90% lymphedema patients, lymphedema in early stages could be surprisingly challenging to diagnose, making assistant methods necessary for early detection and confirmation.

STAGING

It's widely accepted that lymphedema progresses through 4 stages. Stage 0 is the subclinical stage where swelling is absent but with impaired lymph transport and possible complaints of discomfort or heaviness. Stage 1 is spontaneously reversible edema that subsides with limb elevation, while the swelling of stage 2 could not be relieved by elevation. Stage 3, also known as lymphostatic elephantiasis, describes nonpitting edema, fibrosis, hyperkeratosis and the aforementioned complications^[2,9].

DIAGNOSTIC TECHNIQUES

Lymphoscintigraphy

Lymphoscintigraphy has been regarded as the gold standard for the diagnosis of lymphedema since its first introduction. It involves the intradermal or subcutaneous injection into the hand or feet of radiolabeled particles usually under the size of 100 nmol/L, such as ^{99m}Tc (Technetium) human serum albumin nanocolloid, ^{99m}Tc sulfur colloid and ^{99m}Tc albumin colloid. Gamma camera systems are applied to capture the radiopharmaceutical emission as it is taken up and transported by the lymphatic vasculature. Lymphoscintigraphy demonstrates the lymphatic vessels efferent from the injected sites and lymph nodes along the pathway. Typical abnormalities include formation of collateral lymphatic channels, asymmetric visualization of lymphatic channels, delayed or asymmetric node uptake, absent or delayed visualization of lymph nodes, unusual visualization of the popliteal or antecubital lymph nodes (compensatory mechanism involving deeper lymph pathways)^[10,11].

Dermal backflow, accumulation of tracer outside the main lymph routes and in cutaneous lymphatics, and lymphangiectasia are considered major diagnostic findings for lymphedema. Other than morphologic-qualitative information, lymphoscintigraphy provides us with quantitative information of the lymphatics. Commonly used parameters consist of TAT (tracer appearance time, the time from injection to the appearance of the tracer in the inguinal or axillary lymph nodes, normally < 10 min) and TI (Transport Index, normally ranges from 1 to 10).

Hassanein *et al.*^[12] in their study including 227 patients (454 limbs) suggested the sensitivity and the specificity of lymphoscintigraphy for lymphedema is 96% and 100% respectively. Early primary lymphedema may result in false-negative lymphoscintigrams so repeat lymphoscintigraphy is recommended^[12]. The recently developed Taiwan Lymphoscintigraphy Staging might provide a new angle of assessing the severity of lymphedema^[13]. Lymphoscintigraphy is also valuable in early detection and treatment selection, especially surgical planning as it allows to seek out possible functional lymphatic vessels for vessels to use for lymphatic-venous anastomosis (LVA). Compared to lymphangiography, the

tracer used in lymphoscintigraphy rarely causes the allergy and pulmonary embolism, so it's safe and relatively minimally invasive.

Despite its distinct advantages, the protocol of lymphoscintigraphy is poorly standardized, such as the amount of the labeled particles and the injection volume, which substantially affect the quantitative parameters and hinders comparisons between studies. Injection site is also one of the major debates. Tartaglione *et al.*^[11] suggested intermetatarsal or intermetacarpal spaces injection, as compared with traditional interdigital area, results in rapid uptake of tracers, improved imaging quality and reduced examination time (average time 4 h reduced to < 1 h)^[11]. Though combined with computed tomography (CT) or SPECT, spatial resolution of lymphoscintigraphy images improves, it is still far from enough and limited for detection of the small lymphatic vessel leaks^[14]. Owing to discontinuous image acquisition, diagnostic events could happen between acquisition points and be missed. Irradiation is the frequent concern raised in many studies. Though, no cutaneous radio-necrosis has been reported, extra precautions still needs to be taken concerning pregnant and breastfeeding women.

Lympho-fluoroscopies

Lympho-fluoroscopies applies fluorescent molecules such as indocyanine green (ICG), methylene blue *etc.* as the imaging agent. ICG lymphography encompasses the subcutaneous injection of ICG, the usual amount being 0.2 mL. Common injection sites include webspaces of the hand or foot, the medial and/or lateral border of the Achilles tendon, the ulnar side of the palmaris longus tendon at the wrist level^[15,16]. Different near-infrared camera devices are used 12-24 h after injection to record the light emitted by ICG thus visualizing the collecting lymphatic vessels. Linear pattern represents normal or mildly impeded lymphatic collector function, while dermal backflow pattern including splash, stardust of diffuse pattern indicates lymphedema. ICG lymphography is deemed to be the most valuable tool for superficial lymphatics imaging. Compared to lymphoscintigraphy, ICG lymphography is not irradiating with similar sensitivity and specificity (97% and 92%^[17]) but superior resolution and at lower cost. Yamamoto *et al.*^[18] suggested in their study when utilizing ICG lymphography to select optimal sites for LVA, the overall lymphatic vessel detection rate, confirmed by intraoperative findings, is 96.1%^[18]. ICG lymphography can be used for early recognition of lymphedema, as some patients without symptoms can still show abnormal images^[19]. However, ICG lymphography is time consuming and operator dependent. It's unable to observe lymphatics where the tissue is thicker than 2 cm, limiting its possible application in the trunk area and obese patients. Quantification might be more difficult compared to lymphoscintigraphy due to the injection of free ICG (the amount, the concentration *etc.*). Potential toxicity in the lymphatic vessels and its persistence after subcutaneous injection raise some concern because of the lack of studies about its side effects.

The fluorescein used in fluorescence microlymphography (FML) is fluorescein isothiocyanate (FITC)-labeled dextran. 0.1 mL of 25% FITC-labeled dextran solution dissolved by 0.9% sodium or potassium chloride solution is injected into the intradermal layer of the forearm, toes or even the face with a tuberculin syringe and a 25-gauge needle^[20]. Under A fluorescent light microscope, a network of lymphatic becomes visible as the dye spreads through the lymphatics. 10 min after injection, the distance between the border of the injection site and the furthest visible lymphatics is measured in four directions. The maximum extension distance in healthy limbs should not exceed 14 mm. Sensitivity and specificity for the 14 mm cut off level is 91.4% and 85.7%^[20]. Sensitivity was higher in the secondary vs. primary lymphedema^[21]. FML could be used near venous ulcers or indurated skin and rarely cause allergy or other major side effects. However, deeper lymphatic vessels cannot be visualized by FML.

Lymphangiography

Lymphangiography applies various contrast medium and imaging systems to depict lymphatic structures. In direct contrast x-ray lymphangiography, liposoluble contrast medium, such as iodine is directly

injected into the lymphatic vessel dyed by methylene blue. It has been abandoned due to its traumatic nature, technical complexity, poor repeatability and unacceptable contrast complications. Based on the uptake of water-soluble non-ionic contrast agents by lymphatics, indirect lymphangiography avoids direct administration of peripheral lymphatic vessels and has less complications, which is considered to be the best way to differentiate between lipedema and lymphedema.

Magnetic resonance lymphangiography (MRL) involves the subcutaneous/intradermal injection of gadolinium-based MR contrast agents, such as gadobenate dimeglumine, gadoterate meglumine *etc.* into the 4 interdigital web spaces of the hand or foot, with 1% lidocaine as anesthetic. Recommended contrast volume is 1 ml for each site. A 3D heavily T2-weighted sequence or a 3D steady-state free precession balanced sequence^[22] is performed to assess the distribution and extent of edema before injection. Then a fat-suppressed T1-weighted 3D spoiled gradient-echo (SPGR) is used for the lymphatic visualization before and after injection. The number of phase acquisitions and interval varies^[22-24]. A 3D workstation with multiplanar reformations, maximum intensity projection reconstructions and the 3D cursor facilitates image analysis. MRL depicts lymphatic channels, lymph nodes and drainage pattern with supplemental information including fat deposition, muscle compartments and limb volume. Bae *et al.*^[24] and Neligan *et al.*^[25] suggested excellent correlation of MRL with lymphoscintigraphy and ICG lymphography respectively. MRL allows for early recognition, full assessment of lymphedema status and surgical planning especially LVA. Compared to lymphoscintigraphy and ICG lymphography, it is free of radiation and depicts deeper lymphatic channels with higher resolution. Though an extra MR venogram or intravenous administration of Ferumoxytol can help differentiate lymphatic vessels from veins, venous contamination could be a major obstacle in image interpretation. Furthermore, MRL is costly and potentially patient-unfriendly, because it requires patients to stay in the prone or supine position for up to 2 h (the examination duration).

Tissue dielectric constant measurements

Tissue dielectric constant (TDC) is proven to be proportional to local skin-to-fat water content. The Moisture Meter D or its compact version transmits a 300 MHz electromagnetic wave into the tissue and displays absolute TDC values or a percentage of local tissue water, after automatically processing reflected signal. It takes no more than 10 s for each measurement point. TDC ratio (TDC affected/TDC unaffected) > 1.26 is considered suggestive of lymphedema by some^[26]. It can be applied in virtually any areas, midline body regions included, for post-treatment monitoring and early detection. However, TDC is influenced by skin thickness, gender, age, body mass index or race^[27], thus comparison between groups should be dealt with caution and diagnostic threshold is still debatable. In a study by Bakar *et al.*^[28] specificity was 94% with only 65% sensitivity^[28].

Bioelectrical impedance spectroscopy

Bioelectrical impedance spectroscopy (BIS) utilizes a low frequency current to measure electrical resistance (R_0) of local tissue, which inversely proportional to the volume of extracellular fluid volume. For unilateral lymphoedema, the index $R_{0\text{unaffected}}/R_{0\text{affected}}$ is commonly used, the larger the ratio the greater the differences in excess extracellular fluid between limbs. Diagnostic cut-off values varies for non/dominant limbs due to natural asymmetry. The R_i/R_0 ratio is the widely accepted BIS index for bilateral lymphoedema, which R_i means the resistance of the unaffected body region with similar tissue composition as the region of interest. BIS examination only takes a few seconds and rarely causes adverse effects. It is uninfluenced by BMI and reliable in predicting onset up to 10 months prior to clinical manifestation^[29,30]. Sensitivity and specificity for BIS were 64% and 100%, respectively^[31]. However BIS's less sensitive in diagnosing fibrotic lymphedema and breast or trunk measurement is limited. Extra caution should be taken when it comes to patients with pregnancy, cardiac pacemaker or other implanted medical devices.

Table 1. Comparison of different diagnostic techniques

	Sensitivity	Specificity	Advantages	Limitations	Current clinical use
Tape measurement	/	/	Easy to conduct	High inter-/intra-observer variability Poor reproducibility	Therapeutic monitoring Full assessment Experiment
Water plethysmography	/	/	The most accurate measurement of limb volume	Cumbersome set-up Complex measurement protocol	
3D photography	/	/	3D reconstruction image of limb Automatic analysis	High cost	Therapeutic monitoring Diagnosis
Lymphoscintigraphy	96%	100%	Morphologic-qualitative and quantitative assessment of lymphatics	Irradiation Poorly standardized protocol	Diagnosis Surgical planning
ICG lymphography	97%	92%	Valuable superficial lymphatics imaging Low cost	Time consuming (12-24 h) Operator dependent Limited to superficial lymphatics	Surgical planning Early diagnosis
FML	91.4%	85.7%	Time saving (10 min) Applicable to any body regions	Limited to superficial lymphatics	Diagnosis
MRL	/	/	Full assessment of lymphatics and soft tissue High resolution No Irradiation	High cost Time consuming (2 h)	Diagnosis Surgical planning
TDC Measurements	65%	94%	Time saving (10s/measurement point) Applicable to any body regions	Lack of diagnostic threshold due to population variation	Early diagnosis
BIS	64%	100%	Time saving (a few seconds) Uninfluenced by BMI	Reduced sensitivity in late stage lymphedema	Early diagnosis
Ultrasonography	/	/	Central lymphatic channel assessment Ruling out venous cause	Limited measurement range	Supplementary assessment
PET lymphangiography	/	/	Rapid visualization of lymphatics	/	/
Genetic screening	/	/	Early detection	/	/

FML: fluorescence microlymphography; TDC: tissue dielectric constant; MRL: magnetic resonance lymphangiography; BIS: bioelectrical impedance spectroscopy; ICG: indocyanine green; PET: positron emission tomography

Others

CT and magnetic resonance imaging can detect the characteristic honeycomb pattern and the thickening of the subcutis in lymphedema. Ultrasonography rules out edema caused by venous thrombosis or reflux disease. Furthermore, high resolution ultrasonography helps assess central lymphatic channel, such as thoracic duct, the diameter of which is proven to significantly decrease in lymphedema^[32]. We retrospectively analyzed the data of all patients with lymphedema treated in our Medical College Hospital, Department of Lymphedema Treatment Center from September 2015 to January 2017. Patients who had received ultrasound of the thoracic duct were included. A total of 14 patients with lower extremity lymphedema were included. All 14 patients who underwent thoracic duct ultrasonography without lower limb arterial or venous thrombosis met the conditions. There were 5 men and 9 women, aged 15-70 years. All 14 patients had lymphedema in the lower extremities: 5 with left lower extremity lymphedema, 6 with right lower extremity lymphedema, and 3 with both lower extremity lymphedema. Of the 14 patients with lymphedema examined with ultrasound, 6 had a normal thoracic duct diameter and 8 had an abnormal thoracic duct diameter. Ultrasound analysis of the thoracic duct showed that the average inner diameter of the thoracic duct was 2.21 ± 0.15 mm in the six patients with a normal TD and 1.99 ± 0.33 mm in the patients with an abnormal thoracic duct.

Positron emission tomography (PET) lymphangiography with ⁶⁸Ga-labeled NOTA (1,4,7-triazacyclononane-N,N',N''-triacetic acid) with truncated Evans blue (NEB) (⁶⁸Ga-NEB PET) allows for rapid visualization of lymphatic vessels. Long *et al.*^[33] suggested ⁶⁸Ga-NEB PET combined with MRL shows significant advantages over ^{99m}Tc-SC lymphoscintigraphy with MRL in microsurgery preoperative evaluation^[33].

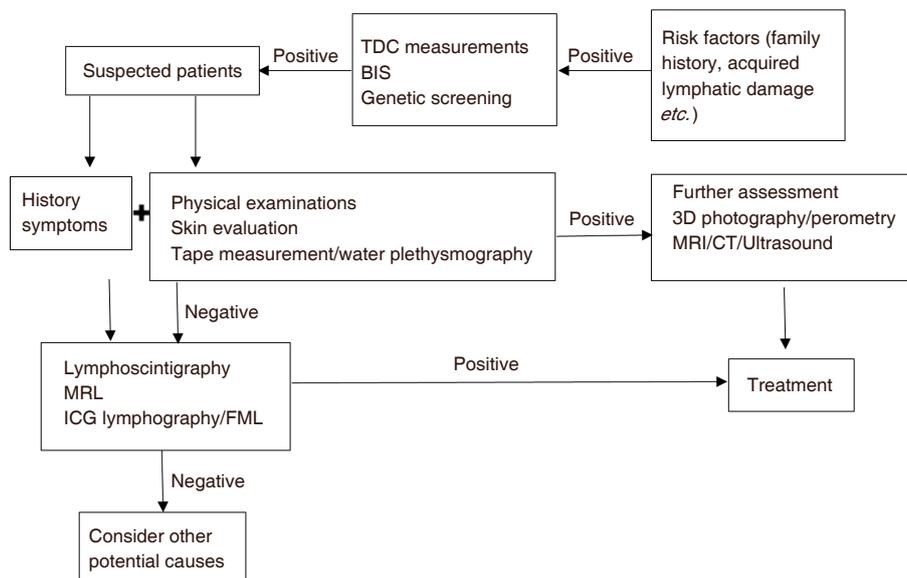


Figure 1. Diagnostic algorithm for lymphedema. FML: fluorescence microlymphography; TDC: tissue dielectric constant; MRL: magnetic resonance lymphangiography; ICG: indocyanine green; CT: computed tomography; BIS: bioelectrical impedance spectroscopy; MRI: magnetic resonance imaging

FOXC2, *GJC2*, *CCNE1*, *SOX18* and *FLT4* gene mutations have been known to be related to primary lymphedema^[9], while *GJA4*^[34], *GJC2*^[34] and *HGF/MET*^[35] mutations correlate with secondary lymphedema. As genomic medicine develops, genetic screening for patients at risk might assist in early detection of lymphedema for the foreseeable future.

CONCLUSION

Since each diagnostic technique has its own pros and cons [Table 1], there's no consensus on how to properly diagnose lymphedema. Adjusting to patients' conditions and clinic facilities, practitioners should choose and combine these diagnostic tools flexibly. Figure 1 demonstrates a potential diagnostic algorithm for lymphedema recommended by the authors.

DECLARATIONS

Authors' contributions

Conceived the structure of the review: Liang ZY

Wrote and revised the paper: Liang ZY, Long X, Yu NZ, Huang JZ

Read and approved the manuscript: Liang ZY, Long X, Yu NZ, Huang JZ

Availability of data and materials

Not applicable.

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None.

Conflicts of interest

Long X is a first-author in one of the referenced papers; Long X, Yu NZ and Huang JZ are co-authors in one of the referenced papers.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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