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Review

Current techniques for lymphatic imaging: State of the art and future perspectives

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Abstract

Techniques for lymphatic imaging are aiming at accurate, simple and minimal-invasive approaches with less side-effects and repetitive application. Limitations are emerging in conventional techniques, and new techniques have shown their advantages in high resolution and sensitivity as well as transcutaneous imaging. In the present review, these techniques and their applications are reviewed and elucidated, aiming at a better understanding of recent advancements and current trends of lymphatic imaging as well as promising techniques for future research.

Keywords: Lymphatic imaging; Near-infrared; MRI; Lymphography; Radiocolloid; Blue-dye

Introduction

The lymphatic system reflects a complex network of lymphatic vessels (LVs), lymph nodes (LNs) and lymphatic organs involved in the transportation of lymph via lymphangions and valves as the basic units of lymphatic vessels. Diagnostic and therapeutic approaches to the lymphatic network are required for both benign and malignant diseases. Currently, there are still limitations in understanding basic mechanisms and concrete structures. These limitations subsequently impact exploration of lymphatic functions, diagnosis of lymphatic diseases and controlling of cancer metastasis negatively.[1] Lack of effective and intuitive methods for imaging in vivo lead to these limitations.

To assess the lymphatic system clinically, imaging techniques are frequently applied. Previously, techniques such as lymphangiography, lymphography with a dye, lymphoscintigraphy, computed tomography (CT), and ultrasound (US) have been employed, but none is able to fully satisfy clinical requirements.[2] Recently, new techniques such as magnetic resonance imaging (MRI), ^{18}fluorodeoxyglucose-positron emission tomography (^{18}FDG-PET)/CT, single-photon emission computed tomography (SPECT)/CT, contrast-enhanced ultrasound (CEUS) and near-infrared (NIR) imaging using Indocyanine green (ICG) have been reported to provide more accurate and detailed information on the lymphatic system.[3]

In order to critically review this emerging field for diagnostic and therapeutic approaches, this article analyzes advantages and disadvantages of current lymphatic imaging techniques and their fields of application. Finally, the requirements for future modalities and the need for further studies are discussed.

Techniques of lymphatic imaging

Direct techniques

Lymphangiography

In lymphangiography, the iodinated contrast agent (ICA) is directly injected into lymphatic vessels, which is the major difference compared to indirect lymphatic imaging

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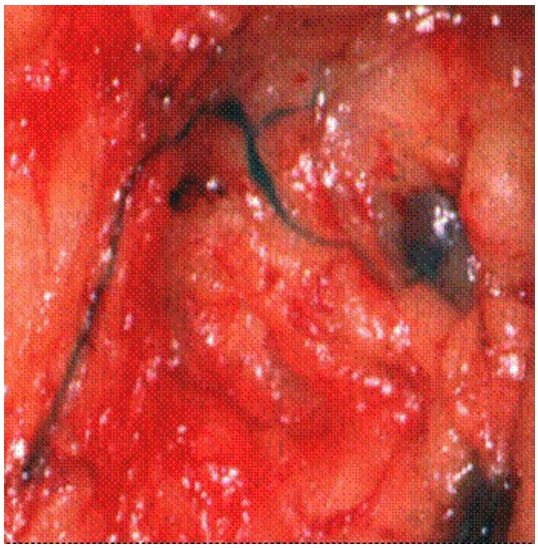

Figure 1. Indirect lymphography guided by a blue dye.

techniques. Lymphatic structures and functions can be observed until the amount of ICA is insufficient.[4] ICA may stay in the body for months to years, which allows long-time follow-up but makes repetitive injections impossible. Due to the invasive and technically difficult cannulation as well as several rare but life-threatening complications, such as contrast-induced nephropathy,[5] lymphangiography has been eliminated in most areas.

Indirect techniques

Lymphography

The indirect lymphography refers to a contrast agent or dye which drains to the lymphatic vessels after being injected interstitially (Fig. 1). The various indirect lymphography techniques are characterized by different modalities which are applied for detection and visualization. While some modalities allow transcutaneous imaging, others require incision and navigation during tissue dissection. Further measures are real-time imaging with or without the application of X-rays. Indirect lymphography allows easier and less time-consuming image generation without further procedures. Furthermore, it avoids side effects of the ICA. Various measures are displayed in Table 1.

Lymphoscintigraphy and single-photon emission computed tomography/computed tomography (SPECT/CT)

Lymphoscintigraphy is the most commonly used indirect lymphatic imaging technique (Fig. 2). Its principle refers to introduction of radioactive tracers and detection with external radiation detectors. The most frequently used tracer is ^{99m}-technetium (^{99m}-Tc) which has a relatively short half-life (6 h) and emits low-energy photons (98.6% at 140.5 KeV, 1.4% at 142.6 KeV).[6] Scintillation cameras are used to form two-dimensional (2D) projection images of radioactive tracer distribution in order to observe lymphatic structures and function. Compared to lymphangiography, the absorption of radioactive tracers is more physiological in lymphoscintigraphy. There is no direct damage to lymphatic vessels, it is easy to repeat, and its sensitivity is much higher.[7] However, its resolution is very poor and the accurate location of lymphatic vessels and nodes cannot be identified because of blurry 2D images. Detailed in vivo navigation to the LN is usually performed by a hand-held probe. There is also an exposure to radiation which requires special radiation protection measures and special waste policies. All together, the technique has limitations with reference to the current requirements for lymphatic imaging in modern clinical practice.

Conventional SPECT is the three-dimensional (3D) reconstruction of multiple-angle projections of radioactive tracers. Although it offers more information than lymphoscintigraphy, its spatial resolution is not satisfactory (1–2 cm).[8] It cannot provide anatomic details, leading to considerable uncertainties in localizing radioactive tracers as well as making correct diagnoses and proper therapy plans.[9] In SPECT/CT, in contrast to the functional SPECT, the relatively precise anatomical location is visualized by CT, and the attenuation can be corrected automatically. A combined 3D reconstruction of SPECT and CT can provide a 360° view of the whole area of interest, which is important in the clinical mapping of LNs.

Table 1
Techniques of lymphatic imaging and their characteristics.

Techniques	Lymph vessels location	Lymph nodes location	Real-time generation	Transcutaneous	Radioactive	Relatively complex equipment	Hand-held
Lymphangiography	+	+	−	+	+	+	−
Blue dye Lymphography	+	+	−	−	−	−	+
Lymphoscintigraphy	−	+	−	+	+	+	+
SPECT/CT	+	+	−	+	+	+	−
^{18}FDG-PET/CT(MRI)	−	+	−	+	+	+	−
MRL	+	+	−	+	−	+	−
CEUS	−	+	−	+	−	−	+
NIR technique	+	+	+	+	−	−	+

SPECT: single-photon emission computed tomography; CT: computed tomography; ^{18}FDG-PET/CT: ^{18}fluorodeoxyglucose-positron emission tomography; MRI: magnetic resonance imaging; MRL: magnetic resonance lymphography; CEUS: contrast-enhanced ultrasound; NIR: near-infrared.

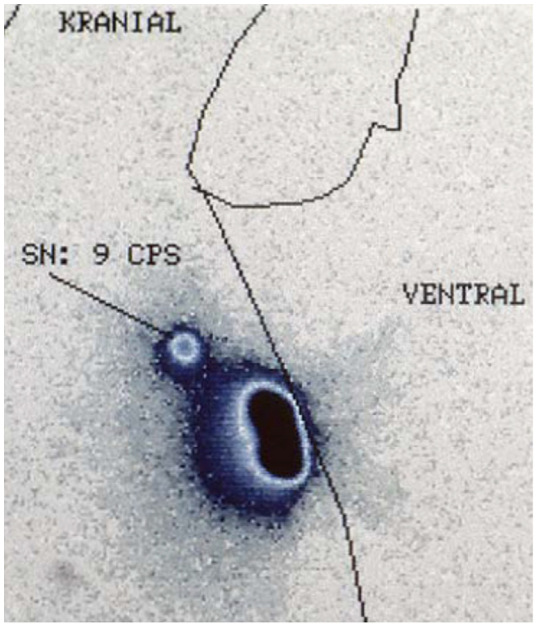

Figure 2. Lymphoscintigraphy using a radionuclide.

In conclusion, lymphoscintigraphy is an old but still important technique for lymphedema diagnosis. SPECT/CT improved visualization of sentinel LNs, but its radiation potentially limits its further employment.

^{18}Fluorodeoxyglucose-positron emission tomography/computed tomography (^{18}FDG-PET/CT) and ^{18}fluorodeoxyglucose-positron emission tomography/magnetic resonance imaging (^{18}FDG-PET/MRI)

PET visualizes the glucose metabolic activity by analyzing the intensity of positron emission. ^{18}FDG is the most commonly PET tracer applied clinically; it is a radioactive modification of glucose by replacing an oxygen molecule with ^{18}fluorine, which emits two photons of 511 KeV and decays to stable ^{18}oxygen.[10] After injection, it is transferred into cells where it cannot be further metabolized after it is phosphorylated to ^{18}FDG-6-phophate,[11] leading to the cellular accumulation of ^{18}fluorine. PET locates the event and analyzes the deposited energy by photons. Most malignant cells can be distinguished from benign due to their much higher rate of glycolysis, except those with a normal or low rate of metabolism, such as in mucinous carcinoma.[12]

PET allows metastases detection in LNs with normal size. However, its primary limitation is the relative low spatial resolution image and lack of an appropriate anatomical definition.[13] PET/CT overcomes those disadvantages by complementing the functional PET with high-resolution CT image on the same axis and correcting attenuation automatically. It has been demonstrated that ^{18}FDG-PET/CT is more accurate than both CT and PET in lymph node staging.[14] Recently, in a preclinical research ^{18}FDG-PET has been integrated to MRI.[15] MRI provides high spatial resolution (3 mm) images combined with high soft tissue contrast without radiation exposure, and ^{18}FDG-PET/MRI has a high sensitivity in detecting LN metastases. The combination is promising and ^{18}FDG-PET/MRI may have an increasing impact on lymphatic imaging in the future. In spite of being complex, time-consuming and expensive; ^{18}FDG-PET/CT plays a significant role in nodal staging.

Magnetic resonance lymphography (MRL)

MRL provides detailed anatomical and functional information with high contrast and spatial resolution, especially in soft tissue imaging. However, the detection of malignant LNs with non-enhanced MRL mainly depends on their size but not the density.[16] Contrast agents[17] have been employed in MRL to decrease the T_1 or T_2 relaxation rate of protons in certain tissues, such as LNs, in order to increase the contrast between them and their surroundings. Ultrasmall super-paramagnetic iron oxide (USPIO, $<$50 nm), which is typically composed by polymeric coatings, such as carboxymethylated dextran, and a core of Fe_3O_4 nanoparticle, can be used as contrast agent. USPIO is specific for the reticuloendothelial system (RES).[18] The accumulation of UPSIO in the RES creates local field inhomogeneity which leads to shorter T_2 relaxation time. Malignant LNs, which mainly consist of tumor cells, do not take up UPSIO because of lacking RES activity. Available USPIO preparation in Europe is Feraheme® (ferumoxytol), which is US market approved.

Compared to the other contrast agents, UPSIO can distinguish malignant LNs from non-affected LN, which in turn largely increases the sensitivity of detection by patient-to-patient analysis; however it mainly offers negative contrast in T_2-weighted or T_2*-weighted images, of which the spatial resolution and signal-to-noise ratios are low. In LNs with micrometastases, it would be difficult to discover small negative contrasted spots, which leads to false negative results.

Enhanced-MRL has shown high specificity for imaging both LVs and LNs. A meta-analysis[19] has shown that its sensitivity and specificity is 90% and 96%, respectively. The advancement in contrast agents and MRI system will further enhance its performance in this field.

Ultrasound (US) and contrast-enhanced ultrasound (CEUS)

Conventional US is a noninvasive technique which accesses the dimension of LNs precisely by offering multilayer and multiangle imaging. Several methods[20] have been reported regarding the discrimination of malignant LNs under conventional US but the outcome seems not quite clinically satisfactory. CEUS has shown its advantages in LNs detection.[21] Commonly used contrast agents are microbubbles typically consisting of a gaseous core, such as sulfur hexafluoride and perfluorocarbon with a

surrounding shell, such as lipids and polymers. After injection, they are drained to local LNs where they are taken up by macrophages there. In high mechanical index setting, microbubbles are quickly destroyed by high acoustic pressure, which is characteristically referred as the acoustic emission (AE) effect.[22] In malignant LNs or LNs with metastasis, the microbubble-uptake of tumor cells is low, which shows perfusion defects or non-uniformity.[23] The clinical safety of UCAs has not been clinically proven yet. Life-threatening complications are reported,[24] and further investigations are needed to conform its safety.

A decisive lymphatic vessel imaging has not been established, so CEUS is limited to lymph nodes, and more research is needed in this new application of CEUS.

Near-infrared (NIR) fluorescence-guided imaging techniques

NIR imaging techniques (Fig. 3) employ fluorescent tracers which emit fluorescence under the excitation of NIR (750–1000 nm) and external fluorescent detectors. ICG, which has been used in determination of cardiac output, testing of liver function and ophthalmic angiography,[25] is a tricarbocyanine dye with properties of NIR absorption and fluorescence emission with its maximum absorption at 778 nm and peak emission at 830 nm in serum.[26] These wavelengths are in the low absorption spectrum of dermis, subdermal tissue and blood (NIR I transparency window),[27] ICG fluorescence has a penetration range up to 30 mm with decreasing definition of the image in deeper tissue.[28] Light emitting diodes and lasers which emit NIR in wavelength around the maximum absorption point of ICG are used as the light source to reduce the background noise produced by other molecules. ICG-Solution (contains 5% sodium iodide) is injected intradermally, subdermally, into the meso or around the tumour, and immediately enables transcutaneous detection of the LVs uptake under real-time conditions. The emitted signal can be visualized by fluorescence imaging systems which typically consists of a band pass filters and charge-coupled device. A pseudo-green color is sometimes added to the image to increase the contrast. In blood, the half-life of ICG is 3–4 min, it is bound to albumin and α-1 lipoprotein, it is exclusively metabolized in the liver and removed from blood to bile in unchanged form. Nearly 90 percents of ICG can be eliminated from blood within 20 min.[29] Generally, the incidence rate of adverse effects is low.[30] The penetration depth of ICG fluorescence may be limited with increase of the dermal thickness. Usually a local enrichment can be detected in case of deeper buried LN. The advantages include high detection rates and sensitivity. It allows preoperative, intraoperative and postoperative imaging of vessels and lymph nodes without radiation, and the devices provide good ergonomy and a flexible deployment.

Primary fields of application

Lymphedema

Lymphedema, which derives from the imbalance between interstitial filtration of plasma and collection of the lymphatic system, is divided into two types: the primary and the secondary lymphedema. The primary lymphedema (PL) is caused by genetic defects and mainly represents a congenital disorder of the lymphatic system. Secondary

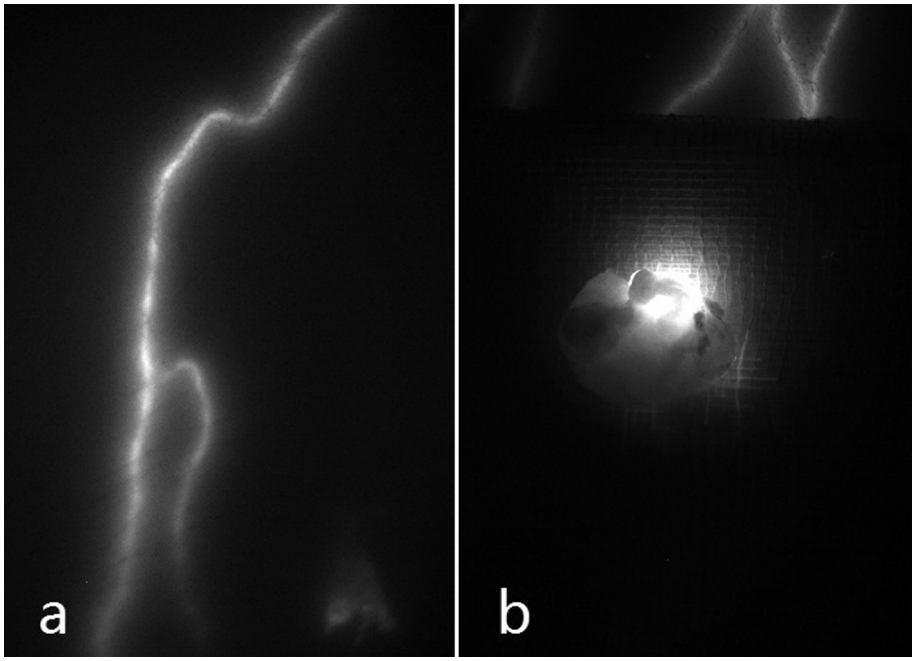

Figure 3. Near-infrared fluorescence guided lymphography using Indocyanine Green (a. lymphography; b, intro-operative view of lymphatic node).

lymphedema (SL) is caused by various damages of the lymphatic system, such as infections, traumas and surgical treatments of malignant diseases. The lymphatic vessel collapses or degenerates and the lymphatic flow is blocked, followed by interstitial accumulation. The most applied imaging techniques for lymphedema are US, lymphoscintigraphy, MRL and NIR-ICG.

US is used as unique imaging technique in the prenatal diagnosis of PL around the 15th week of gestation. Several groups[31,32] have reported that the observation of progressive edema and characteristic skin thickening in fetal feet and lower limbs, as well as edema of umbilical cord plays an important role in the prenatal diagnosis of congenital lymphedema.[33] US is also employed in the diagnosis of filariasis, by detecting the characteristic filarial dance sign (FDS),[34] which indicates the nest of adult nematodes. US detection is safe, rapid and repeatable, allows long-term monitoring of the drug treatment of filariasis.[35]

Lymphoscintigraphy is widely used in the diagnosis of lymphedema.[36] Quantitative and semi-quantitative lymphoscintigraphy have been performed in newborns and children,[37] which is of importance in revealing preclinical lymphedema. Basically there is no significant difference between its employment in PL and SL. Lymphoscintigraphy is also in preparation of both conservative and surgical treatment as well as for follow-up. Maegawa et al.[38] have classified SL patients into five types according to the abnormal pattern of their lymphoscintigrams. Patients with dermal backflow and lymph stasis but without involvement of inguinal LNs are primarily suggested to receive lymphaticovenous anastomosis.

Gadodiamide-enhanced MRL, which can visualize enlarged and tortuous lymphatic vessels,[39] different abnormalities of LNs[40] and thickening of skin and fat lobules,[41] has been introduced by Liu et al.[42] as a more accurate and sensitive method. The high sensitivity in detecting abnormal lymphatic vessels and LNs provides continuative information. Additionally, the contrast agent has been proofed to be safe even in infants,[43] which is important for early diagnosis of PL.

Evaluation of clinical lymphedema using NIR-ICG has become a simple, minimal-invasive and accurate method to detect superficial functional lymphatic vessels comparing to lymphatic pathology. Ogata et al.[44] have successfully observed lymphatic vessels of SL patients intraoperatively using NIR-ICG followed by lymphaticovenous anastomosis which was performed by NIR lymphography. Compared to lymphoscintigraphy, NIR-ICG has been shown to be equally specific but more sensitive in the diagnosis of SL in the arm according to a comparative study in 112 patients.[45] Concerning the simplicity in applying NIR-ICG, it may be a better choice for intraoperative navigation. A NIR-ICG classification system to define the grade of lymphedema has also been successfully introduced by Mihara et al.[46] For higher spatial resolution and continuative measures, MRL with special sequences and USPIO contrast agents may be of additional use for lymphography. 3 T MRL is recommended to find high-defined separation of lymphatic tissue and surrounding muscles, fat tissue and blood vessels.

Sentinel lymph node biopsy (SLNB)

SLN are deemed having a decisive role in staging regional LN metastasis, and SLNB is widely performed in the treatment of several malignances, such as the breast cancer,[47] the melanoma[48] and the gastric cancer.[49]

Recently, CEUS has been used preoperatively of SLNB as a minimal-invasive method.[50] Giogi et al.[51] suggested that CEUS can act as a filter in melanoma patients that spare unnecessary SLNB. So far, there is no comparative research on SLN detection rates between different contrast agents. The choice of contrast agent would be important for SLN detection, and improvements are needed to increase the sensitivity of this method.

Lymphoscintigraphy has been shown to be highly sensitive in cases in which the pattern of lymph drainage is relatively simple and stable. However, in some studies its sensitivity was poor due the aberrant drainage patterns.[52] Accurate anatomical location was provided by SPECT/CT in which the SLNB could be planed more specificity. The operation time for SLNB after SPECT/CT was significantly shorter than with lymphoscintigraphy, and the esthetic outcome was better after SLNB with SPECT/CT because the incision was planned more precisely.[53] NIR-ICG has emerged as a simple and accurate technique for SLNB recently, and has been increasingly used clinically. Murawa et al.[54] demonstrated superiority of NIR-ICG over radiocolloid in breast cancer. Hirche et al.[55] have shown that SLNB with NIR-ICG has a significantly higher detection rate (83%) than the combination of radionuclide and blue dye (75%) in anal cancer. The group[56] also has reported a high rate of SLN detection (97.7%) in breast cancer and colon cancer (96%).[57] Small doses of ICG down to 500 μM have been validated by Van der Vost et al.[3] In summary, the studies with an increasing number of patients indicate that NIR-ICG is a good candidate for SLNB, and studies with larger number of patients are still needed to present more accurate results. MRL can provide additional information due to spatial resolution and exclusive accumulation in the RES of non-malignant lymphatic tissue with limited evidence.[18,19]

Conclusion

Current lymphatic imaging techniques are mainly used for visualization of lymphatic vessels and lymph nodes, aiming at accurate, simple and minimal-invasive procedures with less side-effects and repetitive application. While some modalities are restricted to lymphatic vessel imaging or LN imaging, other modalities provide both approaches. Radiation protection measures and special waste

policies can be spared out using US, NIR-fluorescence or MRL. The combination of transcutaneous and real-time imaging by NIR-fluorescence reflects an optimized approach. Ergonomic features are addressed by handheld probes. MRL requires further research, but is also promising for high-definition and three-dimensional imaging with US-PIO. The combination NIR-fluorescence imaging and MRL or lymphoscinitgraphy for continuative measures may address most requirements for lymphatic imaging with regard to the currently available techniques.

Conflict of interest statement

All named authors hereby declare that they have no conflicts of interest to disclose.

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