Application of Photoacoustic Imaging for Lymphedema Treatment

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Lymphatics are vessels that circulate throughout the body, similar to blood vessels. However, they are difficult to observe from an anatomical point of view and diagnose by radiology because of their small diameter and the transparency of the lymph fluid flowing through them.

Lymphoscintigraphy and near-infrared fluorescence (NIRF) lymphangiography have been used to observe lymphatic vessels for the diagnosis of lymphedema, which is caused by the congestion of lymphatic flow. However, the image resolution from these modalities is insufficient, and it is often difficult to clearly identify individual lymphatic vessels. Moreover, the information obtained from these modalities is two-dimensional, making it impossible to evaluate the depth or the three-dimensional relationships between lymph vessels and blood vessels. In this study, we implemented a photoacoustic imaging (PAI) system that uses the photoacoustic effect, which is a new imaging technique that can clearly visualize the vascular system, hence also visualizing the lymphatic vessels in detail and in three dimensions.

The principle of the PAI system can be summarized as follows (►Fig. 1):

(1) A pulse near-infrared laser repeatedly irradiates the object to be photographed.
(2) The object absorbs light energy and converts it into thermal energy, causing a slight increase in temperature.
(3) When the temperature rises, the object undergoes a slight thermal expansion, resulting in a volume change.
(4) When a volume change occurs, a feeble ultrasound, called a photoacoustic wave, is generated.
This photoacoustic wave is detected by an ultrasound transducer outside the body to create an image. By receiving photoacoustic waves at multiple points during image construction, it is possible to noninvasively identify the location of light absorbers (hemoglobin and melanin, among others) in the body.\(^5\) The imaging test that uses PAI to depict lymphatic vessels is called photoacoustic lymphangiography (PAL).\(^3\)–\(^5\)

Lymphedema is a disease that causes edema in the limb due to congestion of lymph flow, and lymphaticovenular anastomosis (LVA) through the super microsurgical technique is one of the lymphedema treatments that allows stagnant lymphatic fluid to enter. There are various methods of LVA, and although there have been reports that LVA for improving edema can be performed in efferent lymphatic vessels by few anastomoses, especially in early-stage lymphedema,\(^6\)–\(^7\) surgery with more anastomoses is generally more effective, and multiple LVA is generally recommended.\(^8\)

Herein, we report findings on lymphatic vessels obtained using this new device, as well as their clinical application.

**Methods**

This prospective study was conducted from March 2018 at Keio University hospital. We recruited healthy volunteers and patients with lymphedema, and imaged the lymphatic vessels and veins of their lower extremities.

In this study, we used a PAI-05 device (currently undergoing product development).\(^1\)

This system can irradiate lasers of two different wavelengths, making it possible to distinguish between blood and lymphatic vessels based on the difference in the intensity of the photoacoustic waves obtained from them at each wavelength.\(^1\)–\(^2\)\(^,\)\(^9\) However, the transparency of the lymph fluid makes it impossible to delineate the lymph vessels. Therefore, as a light absorber, we used indocyanine green (ICG; Diagnogreen 0.5%; Daiichi Pharmaceutical, Tokyo, Japan), which has also been used for NIRF lymphangiography. The absorbance of ICG increases at 797 nm and decreases at 835 nm. By contrast, absorbance of hemoglobin does not significantly change between these two wavelengths (Fig. 2A). At 797 nm, the absorbance of ICG is significantly greater than that of...
hemoglobin, allowing lymph vessels to be strongly depicted, whereas blood vessels are depicted more strongly at 835 nm. By noting the difference between the images obtained from these two wavelengths, it became possible to distinguish between the lymph and blood vessels (Fig. 2B). In addition, this imaging allows the three-dimensional visualization of the relationship between the lymphatics and the veins (Video 1). Video 1

360-degree view of the Fig. 2B. One can observe the three-dimensional relationship between lymphatics and veins. (Reprinted with permission from Suzuki Y, Kajita H, Konishi N et al. Subcutaneous lymphatic vessels in the lower extremities: comparison between photoacoustic lymphangiography and near-infrared fluorescence lymphangiography. Radiology 2020;295(2):469–474). Online content including video sequences viewable at: https://www.thieme-connect.com/products/ejournals/html/10.1055/s-0041-1736518.

Similarly, for distinguishing arteries from veins, the 756 nm and 797 nm wavelengths can be used, because of the difference in the absorbance of oxidized hemoglobin and reduced hemoglobin becomes greater at 756 nm wavelengths (Fig. 3).1

These arterial and venous data are clinically useful, e.g., when dissecting the perforator flap. In particular, the subfascial area can be identified; therefore, the vascular data are useful to make the flap thin.10

This study was conducted with the approval of the appropriate local ethics committee. Oral and written informed consent was obtained from all participants.

Results

Imaging by Photoacoustic Lymphangiography

The Lymphatics and Veins in Healthy Individuals

The NIRF showed a linear pattern (Fig. 4A), reflecting good functioning of the collecting lymphatic vessels. By contrast, the PAL more clearly showed the collecting lymphatics (Fig. 4B) along with the veins (Fig. 4C).3,11

The Lymphatics and Veins in Patients with Lymphedema

The figures obtained from patients with lymphedema differed with respect to their lymphatic function. In particular, dermal backflow is a characteristic finding in lymphedema. In addition to NIRF, the dermal backflow can often be observed in PAL in the limbs with lymphedema (Fig. 5A).

Moreover, our device was able to observe the object three-dimensionally; hence, collective lymphatic vessels running under the dermal backflow were also identified. Furthermore, not only was PAL regurgitation continuity from collective lymphatic vessels to precollector lymphatic vessels demonstrated, but the relationship between these lymphatics and veins was also observed (Fig. 5B and C). In addition, PAL can digitally eliminate the dermal backflow from the image. Thus, hidden collective lymphatic vessels that run under dermal backflow could also be observed (Fig. 5D).

However, in patients with advanced lymphedema, the function of the lymphatic vessels is deteriorated, and neither the lymphatic vessels nor the dermal backflow could be contrasted (Fig. 6A–6C).

Application of Photoacoustic Imaging

Preoperative Mapping

PAL was performed before LVA surgery, and lymphatic vessels were well-visualized on the images (Fig. 7A and B).
example, at this incision site, the subcutaneous vein and well-depicted lymphatic vessel were identified in the preoperative images. Moreover, the lymphatic vessels had been confirmed at the depth of this vein (Fig. 7C, Video 2) before surgery. Hence, we were able to identify the lymphatic vessels in the deeper side of this vein. The images obtained preoperatively and the actual intraoperative findings were well matched, and a good anastomosis was performed (Fig. 7D–F).

Video 2

360-degree view of the Fig. 7D. This three-dimensional data revealed the fact that the lymphatic vessel run deeper than veins became clear. Online content including video sequences viewable at: https://www.thieme-connect.com/products/ejournals/html/10.1055/s-0041-1736518.
Postoperative Evaluation of LVA

When the anastomosis was observed with PAL, the findings demonstrated the contact between the lymphatic fluid (ICG) and the blood at the border of the anastomosis. When the anastomosis is obstructed, hemoglobin and ICG signals will not be detected at the border, which is thought to cause a gap between the lymph fluid and blood at the anastomosis. In our case, the lymph fluid and blood were joined, suggesting that the anastomosis was patent (Fig. 8A–C).

Discussion

Lymphatic vessels have historically been studied in detail by anatomical studies of lymphatic vessels in cadavers, using...
mercury, or more recently, lead oxide. However, in living humans, observing lymphatics is extremely difficult because the lymph vessels are tiny and the lymph fluid flowing inside the vessels is transparent. The PAI-05 implemented in this study uses ICG as a light absorber, allowing a clearer evaluation of subcutaneous lymphatic vessels compared with existing modalities.

Our group was the first in the world to successfully capture clear three-dimensional images of a wide range of human lymphatic vessels using PAI. This device has a high resolution (0.2 mm) and is capable of individually identifying multiple lymphatic vessels. Handheld PAI devices have also been applied to lymphedema treatment, but the area that can be imaged was only up to 15 mm in diameter.

Previously, imaging modalities such as lymphoscintigraphy, NIRF lymphangiography, magnetic resonance lymphangiography (MRL), and ultrasonography have been used to observe lymphatic vessels for lymphedema treatment. However, although lymphoscintigraphy can evaluate the entire lower extremity, the details of each lymphatic vessel remain unknown. NIRF lymphangiography has the advantage of evaluating lymphatic vessels in real time and is probably the most commonly used technique for preoperative LVA mapping. However, an NIRF cannot provide information on the depth of the lymphatics. MRL
Table 1 Comparison of imaging modalities for lymphatic vessels

|               | Pros                                      | Cons                                                  |
|---------------|-------------------------------------------|-------------------------------------------------------|
| LS            | Enables complete evaluation of the lower extremities | Difficult to identify details of individual lymphatic vessels |
| NIRF          | Real time imaging                         | Provides only two-dimensional information              |
| MRL           | Detailed resolution and wide imaging range in three dimensions | Smaller lymphatic vessels are not evident at early lymphedema stages |
|               |                                           | Venous contamination may lead to misidentification of lymphatics |
| US            | Simultaneous identification of lymphatics and veins No contrast agent required | Reproducibility issues because accuracy of image rendition depends on the skill of practitioner |
| PAL           | High-definition images with a resolution of 0.2 mm enable simultaneous identification of three-dimensional lymphatics and veins Video recording is possible | Imaging range is limited The device is still in its development stage |

Abbreviations: LS, lymphoscintigraphy; MRL, magnetic resonance lymphangiography; NIRF, near-infrared fluorescence lymphangiography; PAL, photoacoustic lymphangiography.

is expected to develop in the future because of its detailed resolution and wide imaging range in three dimensions, but at present, it has difficulties visualizing small lymphatic vessels; moreover, due to venous contamination, some veins are unavoidably enhanced, which may lead to misidentification of lymphatic vessels. Ultrasonography has been applied to the preoperative evaluation of LVA and is able to simultaneously identify lymphatics and veins, while the other three modalities cannot detect veins in detail. Lymphatic structures can also be visualized if ultra-high-frequency ultrasonography is used. However, lymphatic vessels that can be identified vary depending on the skill of the practitioner, and there is a reproducibility problem.

Compared with these modalities, PAL is useful in LVA, considering its detailed imaging ability to identify lymphatic vessels and to simultaneously depict veins. In preoperative PAL, it is possible to simultaneously visualize the lymphatic vessels and veins to be anastomosed. Moreover, the three-dimensional positional relationship of both lymphatic vessels and veins can be clearly seen, which allowed quick identification of each, leading to efficient LVA. Patients with lymphedema have expanded limb appearance, risk of infection (e.g., cellulitis), pain, and lymphorrhea. The main goal of lymphedema surgery is to relieve the patients’ symptoms and decrease limb circumference of the extremity. To achieve this result, attaining a patent anastomosis is expected in the future.

Conclusion

In this article, we reported the use of PAI to delineate lymphatic vessels and their clinical application. This technique can visualize lymphatic vessels with higher resolution than conventional imaging techniques and in three dimensions. Further development of this technique is expected to play a major role in the treatment of lymphedema.

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Conflict of Interest

PAI-05 that we used in this manuscript was provided free of charge from Luxonus Inc. to Keio University School of Medicine. Dr. Aiso is CEO of the Luxonus Inc. Dr. Suzuki reports grants from ImPACT Program of Council for Science, Technology and Innovation (Cabinet Office, Government of Japan), grants from Grant from AMED, during the conduct of the study. Dr. Kajita reports grants from ImPACT Program of Council for Science, Technology and Innovation (Cabinet Office, Government of Japan), grants from Tobikai Medical
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