Lymph Vessel Mapping Using Indocyanine Green Lymphography in the Nonaffected Side of Lower Leg

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Background: The lymph vessels from the dorsum and the medial ankle ascending to the medial side of the thigh toward the inguinal lymph nodes can be observed by the linear flow using indocyanine green near-infrared lymphography (ICGL). Although anatomical studies have shown the widespread existence of lymphatic vessels throughout the body, ICGL shows little linear flow. We herein report our findings of the course of lymph vessels in healthy lower limbs.

Methods: The unaffected lower limbs of 14 patients who underwent lymphaticovenular anastomosis were evaluated for this study. The results of linear flow without massage obtained using ICGL were recorded using a 3-dimensional camera. The positions of lymph vessels were measured from the baseline, which was drawn from the midline of the anterior thigh to the second toe through the middle point of the patella. The locations of the lymph vessels were analyzed using 3-dimensional images at the following 4 points: 10 cm above the knee, at the lower pole of the patella, at the middle aspect of the lower leg, and at the dorsum of the foot.

Results: The average distance from the baseline to the linear flow at each point was 11.39, 9.82, 4.37, and 0.97 cm, respectively. The linear flow was observed inside of the baseline at a distance equivalent to 27.2%, 30.1%, 14.8%, and 4.4% of the leg circumference.

Conclusions: Lymph vessels were observed extensively in the middle lower leg. In contrast, linear flow was limited to a small area at the other measurement points. At 10 cm above the knee, 62.5% of the observed lymph vessels ran 11–12 cm inward from the baseline. Although these results can be useful when choosing incision sites for effective lymphaticovenular anastomosis, this is a pilot study of 14 patients, and studies on a large number of healthy legs need to be done in future.

(Plast Reconstr Surg Glob Open 2020;8:e2929; doi: 10.1097/GOX.0000000000002929; Published online 24 June 2020.)
PATIENTS AND METHODS

The unaffected lower limbs of 14 patients who underwent LVA for the treatment of unilateral lower leg lymphedema were evaluated (Table 1). In all patients, preoperative lymphoscintigraphy showed no abnormal findings such as dermal backflow or disruption and expansion of the lymph vessels in the unaffected limb (Fig. 1).

Indocyanine Green Near-infrared Lymphography

Use of ICGL to diagnose lymphedema was approved by the Ethical Committee of Osaka Medical College (No. 594). ICGL was performed with the patients under general anesthesia immediately before LVA. After the patients had received 4 injections of 0.2 ml (0.5 mg) of indocyanine green into the lateral and medial ankles and the first and fourth toe web spaces, the lymph vessels were observed using an infrared camera (pde-neo; Hamamatsu Photonics K.K., Hamamatsu, Japan) immediately after the injection without a massage. Images of the lymph vessels were then drawn on the skin using an oil-based pen.

Three-dimensional Measurement of Lymph Vessel Locations

To record the locations of the lymph vessels, images of the lower leg containing the drawn-on lymph courses were recorded using a 3-dimensional (3D) camera (VECTRA H1; Canfield Scientific, Parsippany, N.J.). All patients’ feet were fixed using the same fixture, and all 3D photographs were taken from the same angle. The 3D images were then constructed using VECTRA software (VECTRA H1 camera; Canfield Scientific, Inc., Parsippany-Troy Hills, N.J.). The positions of the lymph vessels were measured from the baseline, which was drawn from the midline of the anterior thigh to the second toe through the middle point of the patella. Measurements were performed using 3D images at the following 4 points: 10 cm above the knee, at the lower pole of the patella, at the middle aspect of the lower leg, and at the dorsum of the foot (first metatarsal–cuneiform joint) (Fig. 2). To minimize errors due to differences in patients’ body shape, the distances from the baseline to the lymph vessels were indicated not only by the measured absolute distance but also by the ratio of the distance to the leg circumference.

Relative position of the lymph vessel (%) = Measured distance (cm)/Circumference (cm) × 100

The position of each lymph vessel in the lateral direction was defined by a negative number, while that in the medial direction was defined by a positive number (Fig. 2).

RESULTS

In every case, multiple linear flows were observed in the unaffected lower limb. Among all 14 cases, the average number of linear flows at each measurement point (10 cm above the knee, at the lower pole of the patella, at the middle aspect of the lower leg, and at the dorsum of the foot) was 1.14 (range, 0–2), 1.14 (range, 1–2), 2.64 (range, 1–6), and 1.5 (range, 0–3), respectively (Table 1). The average distance from the baseline to the linear flow at each point was 11.39 cm (range, 8.61–13.96 cm), 9.82 cm (range, 7.03–13.30 cm), 4.37 cm (range, −6.78 to 11.31 cm), and 0.97 cm (range, −1.58 to 2.76), respectively. The average circumferential diameter at each measurement point was 42.6 cm (range, 32.5–55.0 cm), 32.9 cm (range, 27.5–37.5 cm), 30.7 cm (range, 25.0–37.0 cm), and 21.8 cm (range, 19.5–26.0 cm), respectively. In terms of the ratio of the distance to the leg circumference at each point, the linear flow was observed inside of the baseline at a distance equivalent to 27.2% (range, 21.0%–38.8%), 30.1% (range, 22.5%–40.7%), 14.8% (range, −23.4% to 45.0%), and 4.4% (range, −6.8% to 12.6%) of the leg circumference, respectively. (See figure 1, Supplemental Digital Content 1, which displays graphs that represent the relationship between the number of linear flows and the distance from the baseline, expressed as the ratio of the distance to the circumference of the leg, http://links.lww.com/PRSGO/B413.) (See figure 2, Supplemental Digital Content 2, which displays graphs that represent the relationship between the number of linear flows and the absolute distance from the baseline to each of the 4 measurement points, http://links.lww.com/PRSGO/B414.)

Table 1. Cases

| No. | Sex | Age | Side | BMI | Primary Disease       | First Surgery Year | 10 cm above the Knee | Patellar Lower Pole | Middle Lower Leg | Dorsum |
|-----|-----|-----|------|-----|-----------------------|-------------------|----------------------|-------------------|-----------------|--------|
| 1   | Female | 46  | Left | 21.4 | Ovarian cancer        | 2014              | 1                    | 3                 | 1               | 1      |
| 2   | Female | 68  | Right | 23.5 | Cervical cancer       | 2012              | 2                    | 1                 | 2               | 0      |
| 3   | Female | 68  | Left  | 26.0 | Fallopian tube cancer | 2008              | 2                    | 1                 | 2               | 0      |
| 4   | Female | 57  | Right | 21.0 | Cervical cancer       | 1997              | 2                    | 3                 | 1               | 1      |
| 5   | Female | 74  | Right | 20.2 | Ovarian cancer        | 2012              | 0                    | 1                 | 1               | 1      |
| 6   | Female | 86  | Left  | 25.8 | Cervical cancer       | 2005              | 2                    | 1                 | 2               | 0      |
| 7   | Female | 67  | Left  | 20.3 | Bladder cancer        | 2015              | 1                    | 1                 | 2               | 0      |
| 8   | Female | 73  | Right | 32.3 | Idiopathic lymphedema | —                 | 2                    | 3                 | 1               | 1      |
| 9   | Female | 80  | Left  | 19.3 | Cervical cancer       | 1998              | 1                    | 3                 | 1               | 3      |
| 10  | Female | 69  | Left  | 21.8 | Endometrial cancer    | 2001              | 1                    | 3                 | 1               | 1      |
| 11  | Female | 57  | Right | 28.1 | Endometrial cancer    | 2012              | 1                    | 4                 | 1               | 2      |
| 12  | Female | 80  | Left  | 24.6 | Endometrial cancer    | 1991              | 1                    | 2                 | 1               | 1      |
| 13  | Female | 74  | Right | 29.8 | Cervical cancer       | 2005              | 3                    | 3                 | 2               | 1      |
| 14  | Female | 85  | Right | 25.7 | Endometrial cancer    | 2009              | 2                    | 2                 | 1               | 2      |
DISCUSSION

The result of LVA is dependent on the amount of lymph drainage, which is defined by the pressure gradient between the lymphatic and venous systems. Previous studies have revealed that under normal conditions, the lymphatic pressure is lower than the venous pressure. However, the lymphatic pressure during active contraction far exceeds the venous pressure.\textsuperscript{1,2} The key factors that promote lymph drainage are contraction of the skeletal muscles\textsuperscript{3} and contraction of the smooth muscles of the lymph vessels.\textsuperscript{4}

In this study, we observed the lymph vessels by ICGL immediately after injection. The lower leg was the area in which the highest number of lymph vessels was observed. However, the average number of linear flows in this area was only 2.64. Cadaveric studies of lymphatic vessels have revealed that several lymph vessels run mainly along the great and small saphenous veins.\textsuperscript{5,6} However, our study showed that the lymph vessels observed by ICGL were low in number and concentrated in a limited area. One cause of this difference between cadavers and living bodies may be that ICGL cannot depict lymph vessels deeper than 1.5 cm. However, the results of radioisotope lymphoscintigraphy, which can reveal deeper lymph vessels, were correlated with the results of ICGL.

Another possible cause is the difference in the examination methods between the cadavers and the living bodies. In our study, the lymph vessels were observed without massage or exercise, whereas massage is required to move the ICG\textsuperscript{7} through the vessels in cadaveric studies. In lymphoscintigraphy, lymph flow that reaches the groin region early after injection is considered to indicate a lymph vessel with normal smooth muscle function.\textsuperscript{8} Therefore, lymph vessels observed by ICGL early after injection may indicate lymph vessels with normal smooth muscle function.

In this study, the measurement was performed on the 3D images obtained by using 3D camera. The images can be enlarged, and the measurement was taken several times to improve the accuracy of the measurement. Our results indicate that the middle lower leg was the area in which the highest number of lymph vessels was observed. The linear flow was observed inside of the baseline in 13 cases and outside of the baseline in 1 case. In cases of lymphedema, ICGL often shows linear flow on the lateral side of the lower leg.\textsuperscript{9} Our results indicate that the main route of lymph flow may be diverted from alongside the great

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Fig. 1. A 57-year-old woman with right lower leg lymphedema. The results of preoperative lymphoscintigraphy (right) on the unaffected lower limb show no abnormal findings. The results of ICGL were drawn using a green-colored oil-based pen (middle and left). The result of the ICGL of unaffected limb was correlated with the result of lymphoscintigraphy.
saphenous vein to the lateral side. In contrast, only small areas of linear flow were found at 10 cm above the knee, at the lower pole of the patella, at the middle aspect of the lower leg, and at the dorsum of the foot. Our results also showed that 62.5% of the observed lymph vessels ran 11–12 cm inward from the baseline at 10 cm above the knee. These results suggest that the lymph vessels are most likely to be found with a small incision in this area. On the other hand, the remaining function of smooth muscle depends on the stage of lymphedema. We believe that our results are particularly useful in early stages of lymphedema where smooth muscle function remains.

**LIMITATION**

We observed linear flow by ICGL in the unaffected lower leg. Our previous study on ICGL in the affected limb showed that lymph vessels traveled across the usual lymph territories in some cases. Although the lateral route was observed in the middle lower leg in the present study, the results cannot perfectly reflect the dynamic changes in lymph flow caused by lymphedema.

**CONCLUSIONS**

Lymph vessels were observed extensively in the middle lower leg. In contrast, linear flow was limited to a small area at the other measurement points. Among 4 observation points, 10 cm above the knee is where lymph vessels can be identified with a small incision.

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