Impaired lymphatic function recovered after great saphenous vein stripping in patients with varicose vein: Venodynamic and lymphodynamic results

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Objectives: Venodynamics and lymphodynamics may interact as an inseparable and mutually dependent dual outflow system. This study investigated the effect of surgical treatment on lower limb lymph flow in patients with varicose veins.

Methods: Thirty-nine patients with varicose veins in the lower limb (28 patients with unilateral limb, 11 patients with bilateral limb), who demonstrated great saphenous vein reflux, were investigated with air-plethysmography and indocyanine green (ICG) fluorescence lymphography before surgical treatment and 6 months later. Fifteen healthy volunteers participated in this study as a control. With air-plethysmography, venous volume (VV) and venous filling time were measured. Venous filling index (VFI) was calculated. For ICG lymphography, 0.3 mL of ICG (0.5%) was subcutaneously injected at the dorsum of the foot. After the injection, fluorescent image of ICG dye was traced on real-time video images using a near-infrared camera system. The interval until the dye reached the knee was measured (transit time [TT]) in a standing position, which was previously demonstrated to correlate with the interval measured using dynamic isotope lymphoscintigraphy.

Results: In CEAP clinical stage venous disease, TT in patients with C4–6 and C2–3 was significantly longer than that in the control group (587 ± 97 seconds, 484 ± 82 seconds, 252 ± 29 seconds, respectively, mean ± SD, P < .01). Among all limbs with varicose veins, there were correlations between TT and VV (Pearson r = 0.31, P < .01), between TT and VFI (Pearson r = 0.48, P < .01). All patients underwent great saphenous vein stripping. Six months later, the venous clinical severity score significantly improved with significant reductions in both VV and VFI values. TT 6 months postoperatively was also significantly shorter than that before surgical treatment (501 ± 67 seconds, 340 ± 38 seconds, respectively, mean ± SD, P < .01).

Conclusions: Varicose veins could affect lymphatic function and delay lymphatic flow in the lower limbs. Derangement of lymph flow may correlate with the severity of clinical venous disease and/or the magnitude of venous reflux, which could be reversible with surgical treatment of venous incompetence. (J Vasc Surg 2009;50:1085-91.)

Patients with varicose veins often complain of lower limb edema, which is explained by the increased hydrostatic pressure due to varicular incompetence and reflux.1,2 Because venous insufficiency is both progressive and irreversible, clinical symptoms associated with insufficiency increase in severity. To classify the severity of chronic venous insufficiency (CVI), CEAP classification has been used.3,4 During disease development, the contribution of the lymphatic system has not been well understood. Physiologically, the lymphatic system helps maintain fluid, protein, and osmotic equilibrium around cells and aids in the absorption and distribution of nutrients and disposal of wastes. Microcirculatory homeostasis is maintained under equilibrium in pressure balance between tissue and vascular microcirculation. Because CVI causes an increase in venous pressure and subcutaneous capillary network, the pressure balance between tissue and vascular microcirculation is perturbed. One of the reasons for the difficulty in studying the lymphatic system clinically is the lack of an easy and cost-efficient diagnostic test to assess lymphatic function at bed-side. Although direct contrast lymphography provides accurate images of lymphatics,5,6 the test requires a cumbersome and invasive technique and is therefore seldom performed at present.7,8 Currently, isotope lymphoscintigraphy is regarded as the gold standard for an imaging modality to assess lymph flow.9,10 However, lymphoscintigraphy is a time-consuming and expensive test that obtains low resolution images. Therefore, the test is not suitable for screening of the lymphatic system in daily practice.

Recently, we introduced indocyanine green (ICG) fluorescence lymphography as a novel imaging test to visualize lymph flow.11 With a near-infrared camera system, we can easily detect morphologic changes in lymph vessels in patients with secondary lymphedema. The test is safe and can be performed at low cost. Furthermore, we have succeeded in assessing lymphatic function by measuring the transit time (TT), ie, the interval until dye can be carried from the dorsum of the foot to the knee or groin in healthy volunteers.12 In this study, we investigated the effect of venous insufficiency on lower limb lymph flow in patients with...
With approval of the ethical committee and written informed consent, ICG fluorescence lymphography was performed in our outpatient clinic. 0.3 mL of indocyanine green (ICG: Diagnogreen 0.5%; Daiichi Pharmaceutical, Tokyo, Japan) was subcutaneously injected at the dorsum of the foot with a 27-gauge needle in a standing position. Immediately after the injection, fluorescence images of subcutaneous lymphatic drainage were obtained using an infrared camera system (PDE; Hamamatsu Photonics K.K., Hamamatsu, Japan), which activates ICG with emitted light (wavelength: 760 nm) and filters out light with a wavelength below 820 nm. The light source for emission of ICG consisted of 760-nm light emitted diodes (LEDs), and the detector was a charge-coupled device (CCD) camera. Fluorescence images were continuously observed on the monitor of a laptop computer (LaVie G, Type T; NEC Co, Tokyo, Japan). After injection of ICG at the dorsum of the foot, fluorescent images of ICG dye were traced, and the interval the dye reached the level of midpoints of the Patella was measured as transit time (TT) (Fig 1).

Because the venous insufficiency parameters, VV and VFI with air plethysmography, reflect the effect of venous insufficiency mainly on the calf region, we observed the transit time not at the thigh or groin but at the knee. Under epidural anesthesia, we divided all tributaries of the vein at the saphenofemoral junction following ligation and division of the great saphenous vein at the junction. Superior stripping of the great saphenous vein was performed between the junction and just below the knee. Six months later, both air-plethysmography and ICG fluorescence lymphography were performed again at our outpatient clinic. Venous clinical severity score (VCSS) was assessed pretreatment and at 6 months postprocedure.15 Fifteen healthy volunteers (30 legs) with a mean age of 48.0 years (range 25-75 years) participated in this study as control. Duplex scan to verify that there is no venous insufficiency was performed on healthy volunteers. ICG fluorescence lymphography was performed in the same way as for patients with varicose veins.

**Statistical analysis.** All data were expressed as mean ± SD, and differences in the means between the two groups were assessed using paired Student’s t test. Significances of differences in venous hemodynamics and clinical classifications, lymphodynamics and clinical classifications among groups were determined by the one-way analysis of variance followed by Tukey’s test. Regression correlation was calculated between TT and VV, and between TT and VFI. All statistical analyses were performed with GraphPad Prism (ver.5; GraphPad Software, San Diego, Calif). A probability value less than .05 was considered significant.

**RESULTS**

Before surgical treatment, 13 limbs were assigned to class 2, 25 to class 3, 8 to class 4, 2 to class 5, and 2 to class 6 (Table). In the two CEAP 6 patients, both patients had single ulcer with approximately 1 × 1 cm size. Duplex scanning showed venous reflux at the saphenofemoral junction (SFJ)-great saphenous vein (GSV) in all limbs. In the

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Age (y), median (quartiles)</td>
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<tr>
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<tr>
<td>Male</td>
<td>11</td>
</tr>
<tr>
<td>Female</td>
<td>28</td>
</tr>
<tr>
<td>Varicose vein</td>
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<tr>
<td>Unilateral limb</td>
<td>28</td>
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<tr>
<td>Bilateral limb</td>
<td>11</td>
</tr>
<tr>
<td>Total limbs with varicose vein</td>
<td>50</td>
</tr>
<tr>
<td>CEAP classification</td>
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<tr>
<td>C2</td>
<td>13</td>
</tr>
<tr>
<td>C3</td>
<td>25</td>
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<td>C4</td>
<td>8</td>
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<tr>
<td>C5</td>
<td>2</td>
</tr>
<tr>
<td>C6</td>
<td>2</td>
</tr>
<tr>
<td>Total limbs with varicose vein</td>
<td>50</td>
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</tbody>
</table>

By ICG fluorescence lymphography and serially assessed the improvement of lymph flow 6 months after great saphenous vein stripping in comparison to pretreatment values.

**METHODS**

In the period between March, 2006 and June, 2008, 39 patients seeking varicose vein treatment, who agreed to participate in this study, were investigated by both venodynamic and lymphodynamic studies. Inclusion criteria were: (1) presence of great saphenous vein reflux with CEAP 2–6 findings and (2) absence of previous histories of radiation therapy and major trauma to the lower extremities. The subjects consisted of 11 males and 28 females with a mean age of 60.0 years (range 38-75 years) (Table). All patients underwent duplex ultrasound scan with a 7.5-MHz transducer (LOGIC 500; GE Yokogawa Medical, Tokyo, Japan) and air plethysmography (APG-1000; ACI Medical, Sun Valley, Calif) to assess venous function and venous hemodynamics. Clinical disease severity was graded with the standard CEAP classification according to recommendations of an International Consensus Committee on Chronic Venous Disease.13 With duplex scanning, the test was performed in the standing position. An analysis of the venous systems in the saphenous systems, its junctions, and their varicose tracts were carried out, as well as an analysis of the presence of nonsaphenous reflux. Reflux was defined as a flow in an inverse direction to the physiologic flow with duration greater than 0.5 seconds after provocation maneuver. Patients with reflux at the deep vein (either superficial femoral vein or popliteal vein) of deep vein thrombosis (DVT) were excluded from this study.

Air plethysmography (APG) was performed according to the protocol of Christopoulos et al., and the data obtained by APG were on venous volume (VV), venous filling index (VFI), ejection volume (EF), and residual volume fraction (RVF).14

With approval of the ethical committee and written informed consent, ICG fluorescence lymphography was performed in our outpatient clinic. 0.3 mL of indocyanine green (ICG: Diagnogreen 0.5%; Daiichi Pharmaceutical, Tokyo, Japan) was subcutaneously injected at the dorsum of the foot with a 27-gauge needle in a standing position. Immediately after the injection, fluorescence images of subcutaneous lymphatic drainage were obtained using an infrared camera system (PDE; Hamamatsu Photonics K.K., Hamamatsu, Japan), which activates ICG with emitted light (wavelength: 760 nm) and filters out light with a wavelength below 820 nm. The light source for emission of ICG consisted of 760-nm light emitted diodes (LEDs), and the detector was a charge-coupled device (CCD) camera. Fluorescence images were continuously observed on the monitor of a laptop computer (LaVie G, Type T; NEC Co, Tokyo, Japan). After injection of ICG at the dorsum of the foot, fluorescent images of ICG dye were traced, and the interval the dye reached the level of midpoint of the Patella was measured as transit time (TT) (Fig 1).
surgical procedures, ligation and division of tributaries at saphenofemoral junction and subsequent stripping of superior great saphenous vein between the junction and just below the knee were performed in all the patients. The average number of skin incision was 3.6 ± 1.9, in which the additional incision in the lower leg was made in 22 legs after the superior GSV stripping for phlebectomy. No perforator veins in the lower leg were divided. In six legs, sclerotherapy was performed using 1% polidocanol at 4 weeks after the surgery.

Preoperative venodynamic study. The mean VV rose in step with the progression of clinical severity (89.6 ± 21.0, 111.2 ± 36.6, 147.6 ± 52.5 mL, control, C2-3, C4-6, respectively, P < .01). The mean VFI changed similarly among the groups (1.4 ± 1.1, 4.3 ± 2.8, 7.2 ± 3.7 mL/s, control, C2-3, C4-6, respectively, P < .01) (Fig 1).
There were no significant differences in EV, EF, RV, and RVF among the groups (data not shown).

Quantitative ICG fluorescence lymphography before surgical treatment. After subcutaneous injection of ICG at the dorsum of the foot, fluorescence images can be traced along the medial aspect of the limb to reach the knee, in which lymph propulsion was observed with pulsatile lymph flow (Fig 1). TT was recorded by measuring the time interval. In patients with varicose veins, we often saw the standstill of the ICG dye movement just below the varicose vein cluster in the lower leg. In most of the cases, the ICG dye needed several minutes to cross over the cluster (Fig 2). In CEAP clinical stage venous disease, TT in patients with C4 and C2 was longer than that in control group (587 ± 97 seconds, 484 ± 82 seconds, 252 ± 29 seconds, respectively, mean ± SD, P < .01) (Fig 3, C).

Relationships between venodynamic and lymphodynamic parameters before surgical treatment. Comparison of venous hemodynamic parameters to ICG TT identified that both VV and VFI were positively correlated with the TT (r = 0.31, P < .01, r = 0.48, P < .01, respectively) (Fig 4).

Effect of surgical treatment on venodynamic and lymphodynamic parameters. At 6 months after the surgical treatment, clinical symptoms due to venous insufficiency were eased in all patients as indicated with the improvement of VCSS (Fig 5). On air plethysmography, both VV and VFI at 6 months were significantly decreased compared with those pretreatment (VV: 120 ± 43 mL and 90 ± 30 mL, VFI: 5.0 ± 3.2 mL/s and 1.3 ± 1.1 mL/s, pretreatment, at 6 months, respectively, mean ± SD, P < 0.01) (Fig 6, A and B). On ICG fluorescence lymphography, TT at 6 months was significantly shorter than that

Fig 3. A and B, Preoperative venous hemodynamics (venous volume [VV] and venous filling index [VFI]) measured with air plethysmography and clinical severity (CEAP) before surgical treatment. C, Preoperative lymphodynamics (transit time [TT]) measured with indocyanine green (ICG) fluorescence lymphography (TT) and clinical severity (CEAP). *P < .05.

Fig 4. Linear regression analysis between transit time (TT) in indocyanine green (ICG) fluorescence lymphography and venous volume (VV). Venous filling index (VFI) in air plethysmography in patients with varicose vein.

Fig 5. Box and whiskers plots showing Venous Clinical Severity Score (VCSS). At 6 months after surgical treatment, VCSS significantly improved in patients with varicose vein.
pretreatment (501 ± 67 seconds, 340 ± 38 seconds, respectively, mean ± SD, P < 0.01) (Fig 6, C).

DISCUSSION

Varicose vein is a common disease caused by chronic venous insufficiency (CVI), which causes sustained and progressive symptoms such as edema, pigmentation, and ulcer in the skin. To assess the venous hemodynamics in CVI, air plethysmography (APG) has been widely used for quantitative evaluation of venous dysfunction. Among the parameters of APG, VV represents functional venous volume of the lower limb, and VFI estimates the venous refilling rate of the calf, thereby assessing the overall degree of calf venous reflux. Compatible with the findings of previous studies, both VV and VFI correlated with the clinical severity score of CEAP classification in this study. These parameters as well as CEAP clinical severity score correlated well with ICG TT in fluorescence lymphography, which suggested that derangement of lymphatic function, may be correlated with the severity of CVI, which was compatible with previous reports that demonstrated the severe lymphatic damages in CVI patients, particularly around venous ulcers.

Currently, dynamic lymphoscintigraphy is mainly performed to assess lymph function, in which TT measured from the time-activity curve of scintillation counts at the region-of-interest after isotope injection. In this study, we utilized ICG fluorescence lymphography to quantitatively assess lymph function in patients with varicose veins. As we previously reported, measurement of ICG TT (transit time to the knee [TTk] and transit time to the groin [TTG]) is easy to perform at the bedside in either a standing or lying position. These values strongly correlate with TT on dynamic lymphoscintigraphy. Therefore, measurement of ICG TT could become an alternative method of assessing lymphatic function without using isotopes. In this study, we did not compare the TT between dynamic lymphoscintigraphy and ICG fluorescence lymphography in varicose vein patients because the gamma camera for dynamic lymphoscintigraphy can test patients only in the supine position. However, to investigate the effect of CVI, patients should be tested in a standing position without any exercise. Therefore, we did not perform dynamic lymphoscintigraphy in varicose vein patients. Furthermore, we only measured TTk to assess lymphatic function in this study because we thought that TTk might reflect the effect of venous reflux and changes in the parameters of air plethysmography more sensitively than TTG in patients with GSV reflux.

There has been one study assessing lymphatic function in patients with varicose veins using quantitative lymphoscintigraphy. Two hours after injection, they measured uptake at the inguinal nodes as a percentage of the administered dose in the foot. Although only seven patients (14 limbs with varicose veins) were assessed in that study, it clearly demonstrated a lower uptake of isotope at the inguinal nodes in patients with varicose veins, suggesting that lymph drainage was lower in these patients. With FITC-dextran fluorescence microlymphography, Bollinger et al. reported the obliterated microlymphatics and interruptions of the lymph network in the superficial dermal lymphatics in patients with CVI. These findings became more marked as venous disease became more severe. Our study also demonstrated rough correlations between VV and TT or VFI and TT, suggesting the relationship between venous and lymph dynamics. However, the correlation values were not strong so that larger scale of study is needed to confirm it. Six months later, surgical treatment of varicose veins shortened the ICG TT. Taken together, these findings indicate that varicose veins did affect lymphatics and hamper lymph drainage.

The pathogenesis of CVI related lymphatic dysfunction can only be speculated. Because venodynamics and lymphodynamics may interact as an inseparable and mutually dependent dual outflow system in tissue, the mechanism is complex and homeostasis can be maintained with balance between the two systems. Anatomically, the lymph system runs parallel to the venous system in legs, and the structures and functions of these systems show many similarities. CVI increased venous permeability due to capillary hyperpressure, which is associated with increased infiltration and edema. Under this situation, the lymphatic system functions as a buffer by increasing lymph flow. However, these
buffering mechanisms may be finite. Lymphatic collecting vessels carry lymph by their intrinsic pumping activity. With its contractility and valve function, lymph can be propelled toward proximal regions. However, sustained infiltration forcing lymphatic vessels to carry an overload of lymph may adversely affect the pump function and overwhelm the lymphatic system. This phenomenon was observed in animal ex vivo experiments. McHale and Roddie demonstrated that isolated bovine lymph vessels including five to seven lymphangions showed increased frequency of contraction and stroke volume during an increase of transmural pressure from 1 to 4 mm Hg; however, further increase in transmural pressure decreased the stroke volume in the lymphatic vessels. Similar patterns were also reported by Ohashi et al. These lymphodynamic mechanisms together with structural deterioration in the microlymphatic network may underlie the CVI-associated lymphatic dysfunction. Moreover, real-time observation of ICG fluorescence lymphography demonstrated the stagnant lymph propulsion just under extended varicose veins. Among the superficial systems of the leg lymphatics, several collecting vessels are crossed by the great saphenous vein (GSV) and drain to the groin. Therefore, dilated saphenous vein and/or varicoceal vein of their tributaries may directly obstruct flow through the lymph vessels. In this study, we demonstrated the mutual relationship of venolymphatic insufficiency with both venodynamic (air plethysmographic) and lymphodynamic (quantitative ICG fluorescence lymphographic) assessments. We also demonstrated the recovery of deranged lymph flow once the CVI was corrected surgically. Quantitative ICG fluorescence lymphography may be useful to assess the lymphodynamics in various diseases in daily practice. ICG has been widely used in a variety of clinical situations such as examination of hepatic function, and retinal angiography with minor side effects. The greatest advantages of this imaging technique include its ease and real-time visualization as well as safety. However, for patients allergic to iodine, ICG should be used with special precautions. ICG is removed exclusively by the liver so that it can be used for patients with renal insufficiency.

With application of both ICG fluorescence lymphography and APG to patients, we may further elucidate the role of lymphatics in combination with venodynamic systems. Although stripping of GSV was effective to recover lymph flow, we do not know whether other surgical techniques such as endovenous laser treatment or foam sclerotherapy is equally effective as stripping of GSV. The limitation of our study is that ICG TT only reflects the superficial lymph flow and cannot assess the collateral pathways of lymph in the deeper systems once the superficial lymph systems are permanently damaged. Recently, dynamic MR lymphography is reported to visualize the deep lymph vessels with retarded lymph flow. Therefore, when enlarged deep vein is accompanied near the deep lymphatics, MR lymphography may be a useful modality for the functional assessment of deep lymphatics among the current technique.

In conclusion, varicose veins could affect lymphatic function and delay lymphatic flow in the lower limb. Derangement of lymph flow may correlate with the severity of clinical venous disease and/or the magnitude of venous reflux. Deranged lymph flow could be reversible with surgical treatment of venous incompetence. ICG fluorescence lymphography is useful to quantitatively assess lymphatic function.

AUTHOR CONTRIBUTIONS
Conception and design: NU
Analysis and interpretation: MS, NU, YN, MN, DS, HT, YM
Data collection: MS, YN, MN, DS, HT, YM
Writing the article: MS, NU
Critical revision of the article: NU, HK
Final approval of the article: NU
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Overall responsibility: NU

REFERENCES

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