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NOVEL TRANSLATIONAL METHODS

Lower Extremity Lymphatic Flow/ Drainage Assessment by Indocyanine Green Fluorescent Lymphography in Heart Failure Patients

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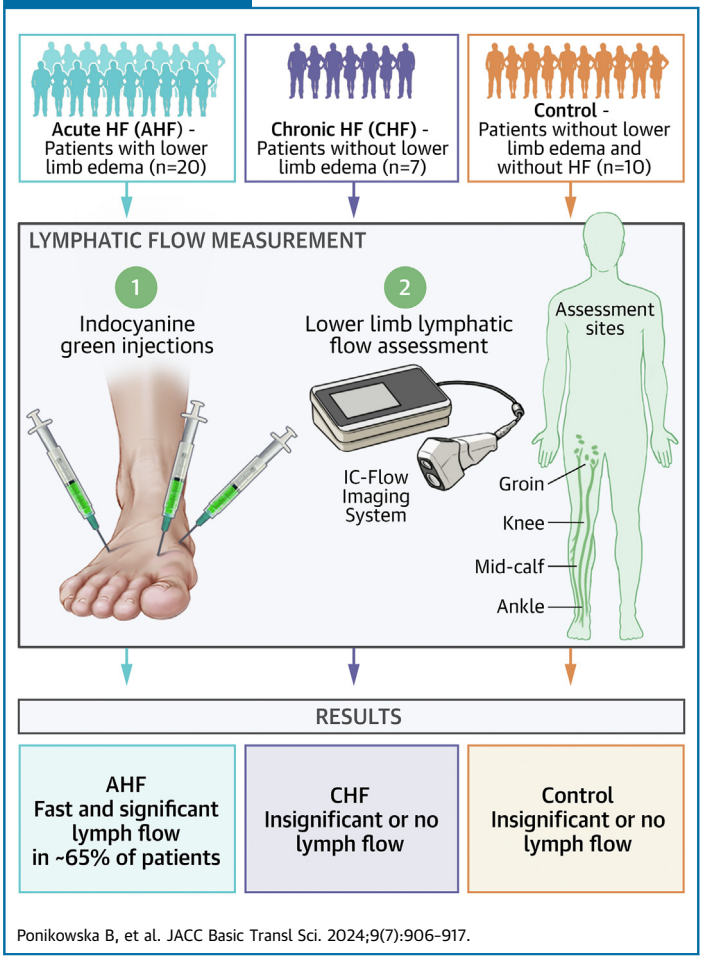
VISUAL ABSTRACT



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HIGHLIGHTS

- **The role of lymphatics in the pathophysiology of congestion in HF has not yet been elucidated.**
- **This study proposes a protocol for visualizing lymphatic vessels and the flow of lymph within them in real time.**
- **The method is minimally invasive and could aid future research and management of congestion in HF.**

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SUMMARY

The purpose of this study was to present a protocol for visualizing lymphatic flow in patients with heart failure (HF) by using indocyanine green fluorescence lymphography. We studied 37 subjects: 20 patients with acute heart failure (AHF) and lower limb edema, 7 patients with chronic heart failure (CHF) without lower limb edema, and 10 control subjects (no HF, no limb edema). All subjects were assessed at rest, and 11 subjects (6 control and 5 with CHF) were assessed again after a 10-minute walk. The lymph flow was visualized in all selected patients without complications. At rest, there was either no lymph flow or minimal lymph flow in all control subjects and patients with CHF, whereas the majority of patients with AHF demonstrated significant lymph flow. This study describes a new method to visualize/assess lymphatic flow in patients with HF, allowing for continuous, real-time tracking of lymphatic flow in the lower extremity. (JACC Basic Transl Sci 2024;9:906–917)

ABBREVIATIONS AND ACRONYMS

AHF = acute heart failure
CHF = chronic heart failure
ESC = European Society of Cardiology
HF = heart failure
ICG = indocyanine green

Congestion is the main cause leading to hospitalization of patients with heart failure (HF), with more than 80% of patients admitted to the hospital due to acute heart failure (AHF) showing overt clinical symptoms/signs of congestion.[1,2] Gradual water and sodium accumulation leads to fluid overload, one of the fundaments of congestion development in AHF.[3] From the clinical perspective, the accumulated extravascular water forms edema, leading to weight gain that often precedes hospitalization.[1,2]

In physiology, a properly functioning lymphatic system is responsible for the constant drainage of extravascular space, keeping the equilibrium between extravascular and intravascular compartments and preventing edema formation. In healthy individuals, the lymph flow in the thoracic duct is estimated to be approximately 2-3 L/d, but can physiologically increase up to 10×, if required.[4,5] Although the potential role of lymphatics in the development of peripheral edema in HF was proposed many years ago, it has been rather rarely investigated, mainly owing to the lack of proper techniques to visualize and assess the system.[6] The early attempts to increase the lymphatic drainage in decompensated HF, such as the 1963 study by Dumont et al,[7] in which 5 patients with congestive HF underwent thoracic duct cannulation with subsequent increase in lymph flow, resulted in significant improvement in the clinical congestion signs. Consequently, recently, more attention has been paid to broadening the understanding of the exact role of lymphatics in the pathophysiology of AHF.[8-10]

Indocyanine green (ICG) lymphography is an imaging technique to visualize lymphatic flow in real time, without radiation exposure. The lymphatic system vasculature in the lower extremities originates from several blind ends and lymphatic capillaries that absorb the interstitial fluid. Thus, the lymphatic vessels surrounding the injection site can absorb the dye and transport it to the inguinal lymph nodes. The method is based on ICG fluorescent lymphography with a dedicated camera to detect the signal from the lymphatics. The use of ICG lymphography in clinical practice is still limited, mainly to sentinel node biopsy procedures, lymphedema diagnosis, and perioperative visualization of lymphatic malformations.[11-13] So far, apart from congenital heart diseases (mainly Fontan circulation, where lymphatics were studied), no studies on the use of ICG in the assessment of the lymphatic system of the lower limbs in patients

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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with acute and chronic heart failure (CHF) have been published.[14-17]

In this study, we used ICG lymphography to visualize, quantitatively describe, and compare the flow in the lower extremity lymphatic vessels in real time in 3 groups of patients—those with AHF with peripheral edema of the lower limbs, patients with CHF and no evidence of peripheral edema, and a control group without heart failure and peripheral edema. Additionally, lymph flow was assessed bilaterally, as well as before and after exercise.

METHODS

The study was conducted in accordance with the guidelines of the Declaration of Helsinki Principles, and informed consent was obtained from all participants. The study was approved by the local Bioethics Committee at Wrocław Medical University. Each participant signed informed consent.

STUDY POPULATION. The lymphatic flow was assessed in 3 groups of patients: those with AHF; those with CHF; and a control group that constituted patients with cardiovascular disease (mainly with hypertension and/or diabetes mellitus type 2) but without HF. Each group was assigned a number: group 1 for patients with AHF; group 2 for patients with CHF; and group 3 for control subjects.

The study groups were selected to demonstrate and compare lymphatic flow in HF patients with potentially different lymphatic activity: those with edema and signs and symptoms of AHF (group 1) to those without edema and a diagnosis of CHF (group 2). We assumed that those 2 distinct states might vary regarding lymphatic flow. The control group (group 3) was included to assess differences in lymphatic drainage between groups 1 and 2 and individuals without HF. Given that some pharmacotherapy agents may affect lymphatic flow, to reduce the risk of bias, the control group was chosen from patients with cardiovascular diseases (mainly hypertension and type 2 diabetes mellitus), in whom HF was excluded.[18] To further understand the differences in the lymphatic flow (and its reserve) in patients without edema, those participants (groups 2 and 3) were asked to perform a 10-minute walk.

Inclusion criteria for groups.

Group 1 (AHF patients). Patients in this group had to fulfill 2 main inclusion criteria, that is, to exhibit signs and symptoms of decompensated HF in accordance with the 2021 European Society of Cardiology (ESC) guidelines for the diagnosis and treatment of AHF and CHF,[19] as well as present with peripheral edema of the lower limbs.

Group 2 (CHF patients). Patients had to fulfill the 2021 ESC guidelines definition of CHF[19] and present without peripheral edema.

Group 3 (control patients). Patients who did not have a history of HF and did not have peripheral edema were included in this group.

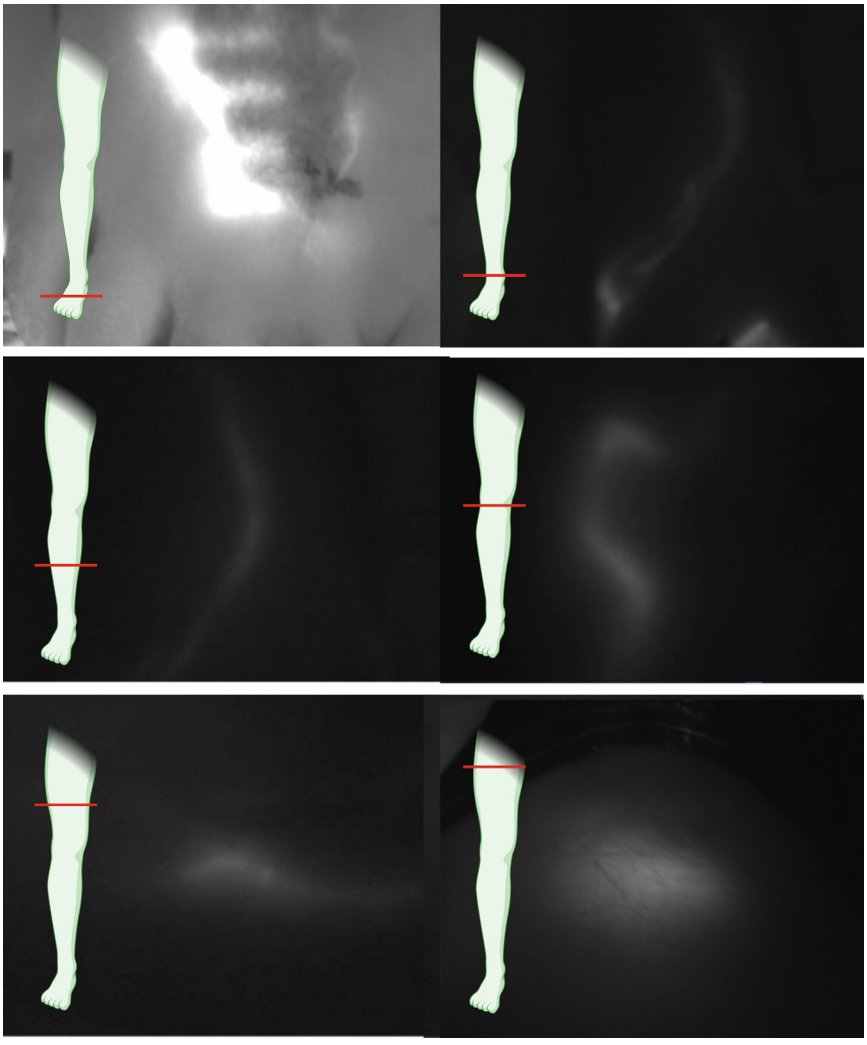
Exclusion criteria for study. The exclusion criteria were universal for all 3 groups and were as follows: Patients with known lymphatic dysfunction or other known non-HF causes of peripheral edema were excluded from the study. Patients permanently immobilized, with venous thrombosis, or with lower extremity ulcers were also excluded.

Sample size. Because there were no reliable data that described the lower limb lymph flow in HF patients (assessed by ICG protocol), the study's sample size was selected arbitrarily.

INDOCYANINE GREEN. The tracer used in this study is ICG Verdye (Diagnostic Green). ICG is an amphiphilic, tricarbocyanine iodide dye ($C_{43}H_{47}N_2NaO_6S_2$, mass = 751 Da), a fluorescent tracer that can be injected subcutaneously with a high safety profile. The dye has been used for diagnostic purposes in medicine for more than 70 years now.[20] ICG can also be administrated intravenously and subcutaneously. ICG has a high affinity to plasma proteins (98% of ICG bounds the proteins), and when given intravenously, it has a short half-life time (3-4 minutes); due to high and rapid liver metabolism, it undergoes hepatic clearance only.[21] ICG fluorescence has high contrast and sensitivity because the near-infrared light used to measure fluorescence makes tissues appear more translucent, probing up to 2-3 cm into the tissue. The tracer is excited by wavelengths between 750 and 800 nm, and fluorescence is observed around the maximum peak of 832 nm.[21,22]

ICG (CAMERA). For the detection of ICG fluorescent signal, we used IC-Flow Imaging System produced by Diagnostic Green. The camera allows to visualize the lymphatic vessels filled with ICG in real time, therefore allowing to follow and visualize the lymph drainage (Figure 1). The camera operator needs to keep an adequate and steady distance between the camera and the skin surface to provide appropriate focus for the images. IC-Flow Imaging System allows for changing the aperture to adjust to the environment in which the measurements are taking place, as well as having the ability to take pictures and record videos during the examination. It is important to emphasize, however, that the narrow penetration depth of about 2-3 cm from the

FIGURE 1 **Predefined Spots on the Lower Extremity**



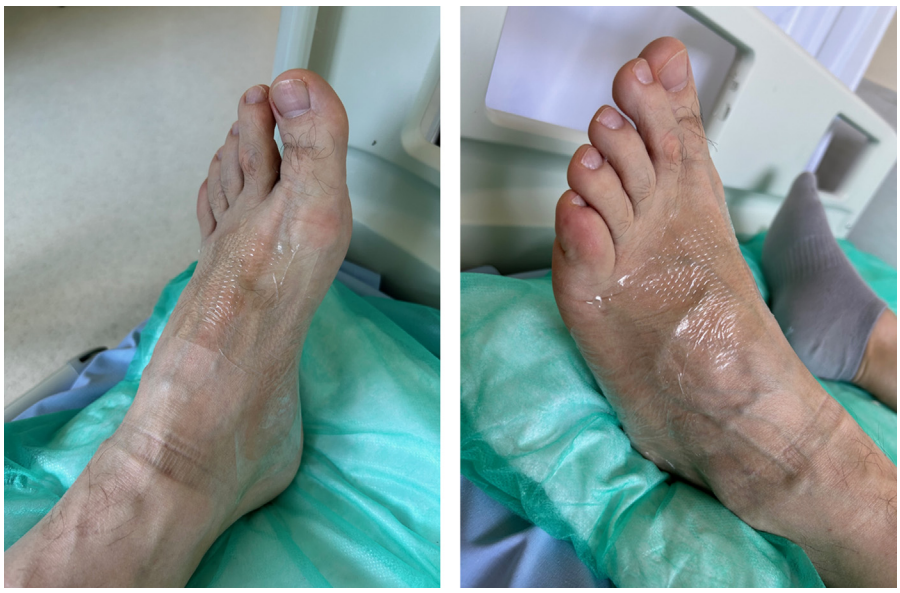
The images recorded with IC-Flow Imaging System show the predefined spots on the lower extremity. Created with BioRender.

skin surface may limit the ability to detect and accurately visualize the ICG signal in patients with more subcutaneous tissue, as well as other parts of the body in which lymphatic vessels are located further from the surface of the skin.

ICG PREPARATION. ICG is supplied in 25-mg vials (5 mg/mL) (Verdye, Diagnostic Green), which contain a powder that needs to be diluted with 10 mL of aqua pro injection before injection. Once the solution is mixed, it forms a plain, green medium that is ready for application. Then, 3 (1 for each foot) separate 1-mL syringes (with 30-gauge needle) containing 0.5 mL of ICG each are prepared.

PROTOCOL OF ICG INJECTIONS AND LYMPH FLOW ASSESSMENT. Because the lymphatic flow is affected by the lower extremity muscle pump, in our protocol, patients were asked to lie down and rest for at least 1 hour before the start of the injection procedure. Patients were examined in a supine position in a quiet room. To allow better lymphatic vessel contrast and visualization, the blinds in all windows were shut during the assessment. Before the ICG injection, the patient's skin was anesthetized by topical application of anesthetic cream (EMLA Cream 5% lidocaine/prilocaine, 25 mg/g + 25 mg/g, Aspen Pharma) in the 3 predefined spots. We decided not to use standard lidocaine subcutaneous injections to mitigate the

FIGURE 2 Injection Sites Prior to ICG Injection



The injection sites with topical anesthetic and dressing applied, prior to indocyanine green (ICG) injection.

potential risk of local lymphatic vessel palsy by the lidocaine injections. The cream was applied to the skin and covered with the dressing supplied by the producer for at least 45 minutes (Figure 2). After that time, the dressing was removed, and the cream was wiped off. The skin was then disinfected and ready for ICG injections. Then, 3 syringes (for each examined foot) with 0.5 mL ICG each were prepared to reduce the delay between the first and the last ICG injection. The stopwatch was set up, and 3 separate subcutaneous injections of 0.5 mL ICG each were performed, making sure the delay between them was minimal. Acknowledging the fact that there are 4 main, independent lymphatic vessel groups in the lower extremity, we injected the ICG dye in 3 predefined spots in each foot (Figure 3).[11] Once all the injections were performed, the stopwatch was started, and the operator changed the gloves to prevent any contamination of the patient's skin with the ICG, which may cause significant artifacts. Then, the time needed for ICG to reach the predefined spots in the lower extremity was measured. After 10 minutes had elapsed since the injection, the distance from the injection site to the furthest point reached by the ICG was measured with a tape measure and recorded.

Patients in group 1 received furosemide in the dosage of 1 mg/kg of body weight intravenously (40-mg bolus and the remaining dose in an intravenous drip) 3 hours before the injection of ICG. We decided to use standardized doses and methods of administering the furosemide to ensure that all patients receive comparable doses of diuretics.[23] This helps reduce the risk of bias related to different diuretic regimens that may lead to different responses and potentially have different effects on lymphatics. The timing for initiating lymphatic flow measurement was strategically chosen owing to the documented peak of diuretic response occurring at the second to third hour after furosemide administration,[23] leading us to conclude that this specific time frame presented optimal opportunities for capturing activated lymphatic drainage.

SELECTED SITES ON THE LOWER EXTREMITY. We have selected the following sites in the lower extremity: ankle; mid-calf; knee; and groin (Figures 1 and 3).

LYMPHATIC FLOW ASSESSMENT. To assess the lymph flow, we have measured the time (in seconds) needed for the lymph (ICG tracer) to reach predefined anatomical locations on the lower extremity (Figure 3) and the distance (in centimeters) that ICG reaches within 10 minutes of injection. In randomly selected patients from group 1, the lymph flow was assessed

FIGURE 3 Sites for ICG Injection and Flow Measurement

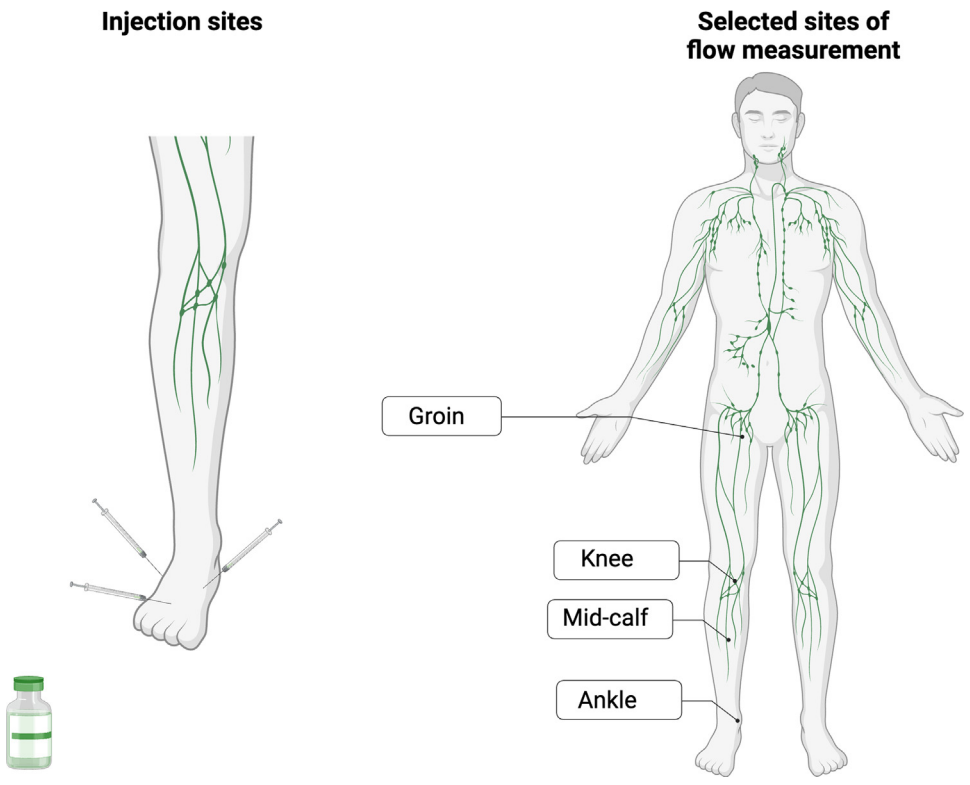


Illustration of indocyanine green (ICG) injection sites (left) and selected sites of flow measurement (right). Created with BioRender.

bilaterally. And in randomly selected patients from groups 2 and 3, the lymph flow was assessed pre- and post-10-minute exercise (walking).

The 10-minute walking exercise was chosen on the assumption that it could effectively activate the muscle pump, but it was selected arbitrarily owing to the absence of any validated exercise test for lymphatic drainage activation in HF.

STATISTICAL ANALYSIS. Continuous variables with a normal distribution are described using mean ± SD, variables with skewed distribution are described by median (Q1-Q3), categorical variables are presented as count and percentage. The normality of data distribution was determined by the Shapiro-Wilk test. The drainage between both legs was compared using an intraclass correlation coefficient with a 95% CI. The comparison of lymph flow between the study groups was assessed by the Mann-Whitney *U* test. The number of patients with lymph flow was compared using the Fisher exact test. A value of $P < 0.05$ was considered statistically significant. Statistical analyses were performed using STATISTICA (version 13.3, StatSoft).

RESULTS

There were 20 patients in group 1, 7 in group 2, and 10 in group 3. The study population was predominantly male, with male participants constituting 80% of both group 1 and group 3. Group 2, however, did not include any female participants. The mean body mass index in groups 1-3 were 29 ± 6 kg/m^2, 26 ± 2 kg/m^2, and 29 ± 5 kg/m^2, respectively (Table 1).

The mean ejection fraction of group 2 and group 1 patients was 29% ± 13% vs 41% ± 16%, respectively, and the median N-terminal pro-B-type natriuretic peptide was 2,054 (Q1-Q3: 1,801-2,317) pg/mL and 5,438 (Q1-Q3: 4,008-10,808) pg/mL, respectively. More detailed information on each study subpopulation's characteristics is presented in Table 1. Table 2 contains information on signs of congestion in group 1 patients, besides peripheral edema, which was present in all patients.

TABLE 1 The Baseline Characteristics of Examined Patients

	Group 1 (n = 20)	Group 2 (n = 7)	Group 3 (n = 10)
Male	16 (80)	7 (100)	8 (80)
Age, y	71 ± 16	54 ± 17	65 ± 13
Weight, kg	85.7 ± 17.6	84.1 ± 9.9	85.6 ± 13.1
BMI, kg/m^2	29 ± 6	26 ± 2	29 ± 5
BMI range, kg/m^2	20-38	24-30	24-38
Systolic blood pressure, mm Hg	127 ± 29	129 ± 25	143 ± 28
Left ventricle ejection fraction, %	41 ± 16	29 ± 13	51 ± 13
Blood count			
Hemoglobin, g/dL	11.7 ± 2.6	14.7 ± 1.0	14.7 ± 1.2
Hematocrit, %	36.6 ± 7.1	44.3 ± 3.8	44.1 ± 3.2
White blood cells, 10 × 3/μL	7.4 ± 2.6	6.8 ± 2.4	7.4 ± 1.5
Platelets, 10 × 3/μL	215 ± 72	250 ± 75	202 ± 38
Biochemistry			
Serum Na^+, mmol/L	142 ± 4	141 ± 1	140 ± 2
Creatinine, mg/dL	1.41 ± 0.37	1.03 ± 0.19	0.84 ± 0.16
Urea, mg/dL	72 ± 43	26 ± 6	35 ± 12
NT-proBNP, pg/mL	5,438 (4,008-10,808)	2,054 (1,801-2,317)	396 (112-1,994)
Pharmacological treatment			
Beta blockers	16 (80)	7 (100)	5 (50)
Aldosterone receptor antagonists	10 (50)	5 (71)	4 (40)
SGLT2 inhibitors	11 (55)	1 (14)	2 (20)
Angiotensin-converting enzyme inhibitors	14 (70)	4 (57)	7 (70)
Sacubitril/valsartan	2 (10)	1 (14)	0 (0)

Values are n (%), mean ± SD, or median (Q1-Q3), unless otherwise indicated.
BMI = body mass index; NT-proBNP = N-terminal pro-B-type natriuretic peptide; SGLT2 = sodium glucose cotransporter 2.

TECHNICAL SUCCESS OF THE PROTOCOL. All selected patients underwent the procedure of lymphatic drainage assessment without any complications. The lymphatic vessels were visualized in all selected patients.

FLOW ASSESSMENT. Patients in group 1 were assessed at rest only. In this group, 13 patients (65%) had lymph flow (defined as lymph flow beyond the ankle within ≤10 minutes), whereas in the remaining 7 (35%), there was minimal or no flow observed. The median times to reach the ankle, mid-calf, knee, and groin were 85 (Q1-Q3: 45-225) seconds, 162 (Q1-Q3: 120-330) seconds, 300 (Q1-Q3: 180-420) seconds, and 570 (Q1-Q3: 450-775) seconds, respectively. In 12 patients (60%), the lymph reached the groin level during the examination (for more details, see Table 3 and Figure 4). The median distance of lymph flow reached after 10 minutes in the entire group was 49 (Q1-Q3: 10-79) cm.

TABLE 2 Other Forms of Congestion in Patients in Group 1

Ascites	8 (40)
Paracentesis	2 (10)
Pulmonary congestion	13 (65)
Pleuracentesis	5 (25)

Values are n (%).

At rest, patients in group 2 also exhibited no or minimal flow; 3 patients (60%) had the ICG reaching the predefined spot on the ankle, with the median time of reach being 120 (Q1-Q3: 101-135) seconds, and median distance lymph reached after 10 minutes in this group was 5 (Q1-Q3: 5.0-10.0) cm. One patient in this group exhibited flow above ankle level. After an exercise, the median lymph flow was 27 (Q1-Q3: 6.0-32.5) cm.

All patients in the group 3 had no or minimal flow at rest. Five patients (50%) in group 3 had the ICG reaching the predefined spot on the ankle, with a median time to reach the ankle of 120 (Q1-Q3: 100-120) seconds, and the median distance the lymph (ICG) reached after 10 minutes was 5 (Q1-Q3: 0.0-8.0) cm. One patient in group 3 demonstrated lymph flow above the ankle level. After the exercise (10-minute

TABLE 3 The Lymph Flow Assessment at Rest

	Group 1 (n = 20)	Group 2 (n = 7)	Group 3 (n = 10)	*P* Value[a]
Lymph flow at rest[b]	13 (65)	1 (14)	1 (10)	0.021
Distance lymph reached after 10 min, cm	48.5 (10-78.5)	7.0 (5.0-10.0)	5.0 (0.0-8.0)	0.018
Patients with lymph flow reaching				
Ankle	20 (100)	3 (43)	5 (50)	0.002
Mid-calf	14 (70)	0 (0)	0 (0)	0.002
Knee	13 (65)	0 (0)	0 (0)	0.006
Mid-thigh	12 (60)	0 (0)	0 (0)	0.008
Groin	12 (60)	0 (0)	0 (0)	0.008
Time needed to reach, s[c]				
Ankle	85 (45-225)	120 (101-135)	120 (100-120)	0.39
Mid-calf	162 (120-330)	NA	NA	
Knee	300 (180-420)	NA	NA	
Mid-thigh	450 (270-595)	NA	NA	
Groin	570 (450-775)	NA	NA	

Values are n (%) or median (Q1-Q3). [a]*P* value for comparison between CHF and AHF. [b]Defined as lymph flow beyond the ankle within 10 min or less. [c]The median time is presented only in patients in whom the flow reached the predefined point.

AHF = acute heart failure; CHF = chronic heart failure.

walk), the median distance of lymph flow was 21.5 (Q1-Q3: 11.0-38.0) cm.

COMPARISON OF LYMPH FLOW BETWEEN THE GROUPS. Group 1, when compared with group 2, had a significantly higher percentage of patients with lymph flow at rest—65% vs 14%; $P = 0.021$—and longer median distance lymph reached after 10 minutes—48.5 (Q1-Q3: 10-78.5) cm vs 7.0 (Q1-Q3: 5.0-10.0) cm; $P = 0.018$. Analogically, the median distance lymph reached after 10 minutes in group 1 was significantly longer when compared to group 3: 48.5 (Q1-Q3: 10-78.5) cm vs 5.0 (Q1-Q3: 0.0-8.0) cm; $P = 0.002$ (Table 3). The comparison between groups 2 and 3 did not reveal any significant differences in lymph flow. The percentage of patients with lymph flow reaching each predefined point in the lower extremity was significantly higher in group 1; all $P < 0.05$ (Table 3, Figure 4).

COMPARISON OF SIMULTANEOUS LYMPH FLOW IN BOTH LOWER LIMBS. When lymph flow was examined simultaneously in both legs (n = 6), a moderate correlation was observed between the contralateral measurements, with an intraclass correlation coefficient of 0.548 (95% CI: −0.465 to −0.924); $P = 0.12$. This was attributed to the fact that in 1 patient, lymph flow was observed in only 1 leg. When the correlation was recalculated among patients in whom bilateral flow was visualized (n = 5), the intraclass correlation coefficient improved significantly to 0.947 (95% CI: 0.429-0.995); $P = 0.004$. The mean distance reached after 10 minutes was 30 ± 16 cm for the left leg and 27 ± 13 cm for the right leg; p=0.25.

SAFETY. We have not noted any serious adverse events during the ICG injections and protocol execution. The only adverse events that we noted were the pain related to the ICG application and the tracer retention at the place of injection (Table 4).

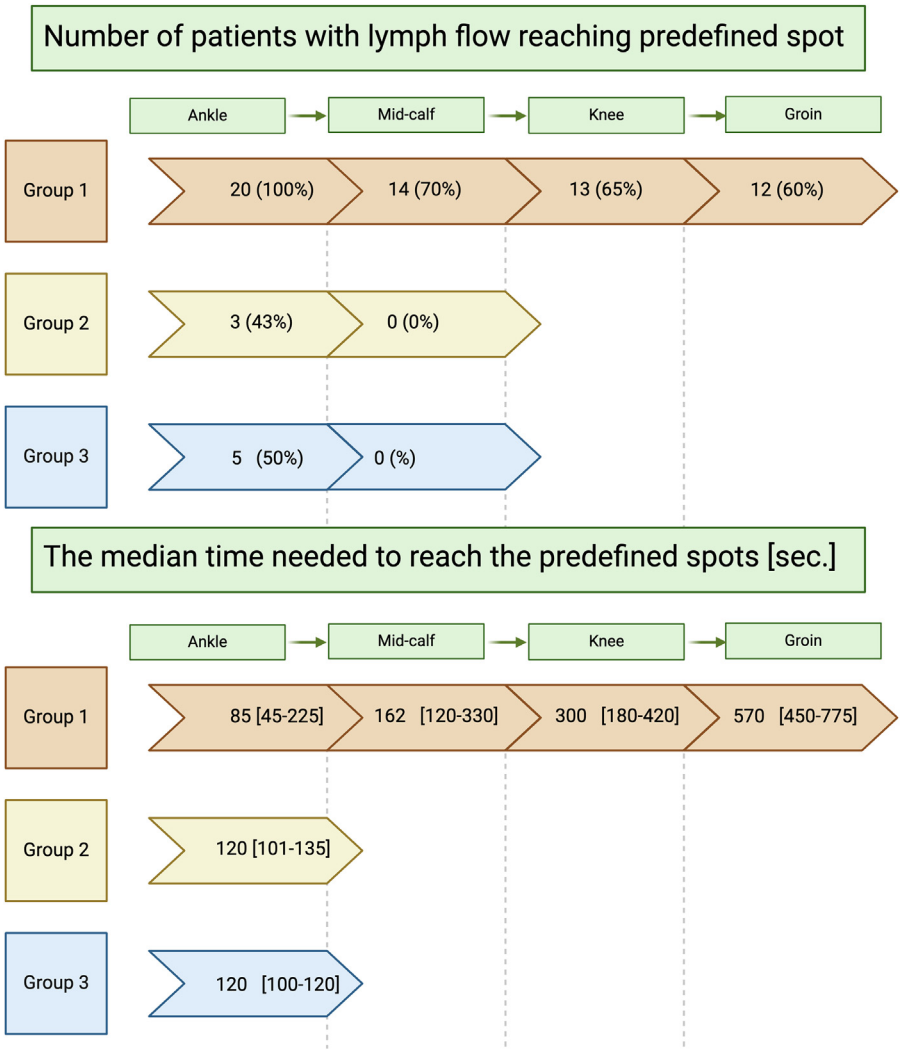
DISCUSSION

We believe there are at least 3 important aspects of our paper. First, we introduce a method for visualizing and quantitative assessment of peripheral lymphatic flow in real time.

Second, we have demonstrated that the protocol is safe, feasible, and may be promising for future research. Third, the application of this protocol in HF patients has revealed variations in lymph flow between patients with AHF (with peripheral edema) vs both patients with CHF (without edema) and control subjects without HF. Interestingly, during rest, a majority of group 1 patients (65%) exhibited noticeable lymph flow, with the tracer reaching a distance of 47 cm within 10 minutes. In contrast, in group 2 and 3 patients, the flow was minimal.

The protocol was created and used in our research laboratory to visualize and assess the lymphatic flow in the lower extremities of patients with HF. This procedure allows for continuous tracking of the lymphatic flow that can be easily assessed, even at the bedside. Whereas the involvement of lymphatic drainage in the pathophysiology of peripheral edema in HF is undisputed, direct confirmation of its role was difficult owing to challenges in visualizing the lymphatic system and drainage. The use of ICG for detecting lymphatic vasculature has been established

FIGURE 4 Number of Patients With Lymph Flow Reaching Predefined Spots and Median Time



Flow chart showing the number of patients with lymph flow reaching predefined spots and the median time needed to reach them. Created with BioRender.

for many years. However, in our study, we propose a new protocol that leverages the unique ability of the IC-Flow Imaging System to examine and track (in real time) the lymphatic drainage of patients with decompensated HF.

TABLE 4 The List of Adverse Events Related With the Lymphatic Drainage Assessment

Adverse Event	Severity	Frequency
Pain during ICG injection	Moderate	Very often
Retention of the tracer at the place of injection	Mild	Always

ICG = indocyanine green.

We believe that the proposed protocol of dynamic lymphography in HF could potentially have a practical and scientific use. First, it allows the detection of the lymph flow in the lower extremities and, therefore, differentiates patients with vs without the flow at rest as well as after the activation of the muscle pump. Second, it can also provide quantitative information on the flow that could be used for the assessment of the effects of the interventions targeted to the lymphatic system.

In group 3 patients (without evidence of peripheral edema), we observed only very low lymph flow at rest, which, as we supposed, may reflect physiologic patterns in individuals who do not

TABLE 5 Advantages and Disadvantages of the Proposed Protocol

Advantages
Easy to perform
Real-time, point-of-care visualization of lymphatic anatomy
Assessment of lymphatic flow
Low risk of adverse events
Disadvantages
Time-consuming preparation
Costs of IC-Flow Imaging System and ICG tracer
Pain of subcutaneous application
Retention of the tracer at the place of injection
Cannot be repeated unless the tracer is washed out
Potentially insufficient penetration of the camera 2-3 cm from the skin surface in obese patients
We did not assess the lymph flow in liters per hour, but we can assess the time needed to reach predefined points (ie, knee)

ICG = indocyanine green.

need to drain the interstitial space extensively. The flow significantly increased after a 10-minute walking exercise, which is also important information that could potentially be useful in future studies protocols. A similar pattern of lymphatic flow was observed in patients in group 2 who did not have peripheral edema. In contrast, the majority of patients in group 1 demonstrated much faster and increased lymphatic flow, which may be indirect evidence of the role of the activated lymphatic system in the pathophysiology of lower limb edema. Interestingly, one-third of patients in group 1 (with overt peripheral edema) had minimal or no flow, which is difficult to interpret. These patients need more detailed evaluation because we can only hypothesize that in such cases, the lymph flow can be augmented by muscle pump in some patients and, therefore, might identify different phenotypes of lymphatic insufficiency.

The lymphatic system has become an interesting and promising therapeutic target that may facilitate decongestion in AHF. There is an ongoing study using a device designed to reduce venous pressure at the thoracic duct outlet and, therefore, improve lymph return to the central circulation. The early animal model of the device provided favorable results.[24] Whatever the peripheral lymph flow follows and adjusts to central (thoracic duct) flow remains a subject of future research. Theoretically, the proposed protocol could be used to measure the effect of central lymphatic decompression; however, this needs to be prospectively evaluated.

The ICG injected into the interstitial space penetrates the lymphatic vessels, filling them, enabling localization of the vessels, and detecting the flow within them. It is important to emphasize that this method does not allow for the assessment of lymph flow expressed as a numeric value, for example, in lymph volume over time. When a lymphatic vessel is filled with a tracer, we are not able to evaluate whether any processes are continuously occurring within it. Instead, we can only assess the propagation of the tracer filling the vessels and its movement toward the heart. The other limitation of the protocols on ICG is that the examination cannot be repeated within the hours of the first injection, because one needs to wait for the dye to be washed out of the lymphatic vessels first. Notably, the procedure based on ICG is safe and has several advantages, including that it is easy to perform, allows real-time visualization, and can be performed at the bedside (see Table 5).

STUDY LIMITATIONS. First, the low number of patients examined limits the generalizability of our study. Second, despite the solid pathophysiologic backgrounds supporting the notion that die movement within the lymphatic vessels ICG represents the lymph flow and therefore allows real time, dynamic lymph flow assessment, this notion has not been validated. Because we were unable to predict the lymphatic flow in the examined subpopulations, we could not calculate the sample size of the study population. Only a subset of patients had bilateral lymph flow assessment, and the 10-minute walking exercise used was not validated to result in lymph flow. It would be very interesting to examine whether activation of the muscle pump (by the exercise) could activate the lymph flow in group 1 patients with no flow at rest, but the test was not performed in that group. Furthermore, the mechanisms that impede lymph flow in some HF patients are complex and may include lymphangion dysfunction, lymphatic valve insufficiency, elevated intra-abdominal pressure, and elevated central venous pressure, which prevents the drainage of the thoracic duct into the central circulation. The present study did not examine these mechanisms.

CONCLUSIONS

The protocol presented in this study provides a new method for visualizing and assessing peripheral lymphatic flow. It allows for continuous, real-time tracking of lymphatic flow and enables

examination of all lymphatic drainage pathways in the lower extremity. Although this method has some limitations, including the inability to perform serial flow assessments within a short period of time, it has several advantages and is safe and easy to perform. Overall, this protocol provides a valuable method for lymphatic drainage assessment in patients with HF. It could potentially help to understand the role of the lymphatic system in the pathophysiology of HF and, as a result, could aid in developing new ways of treatment for peripheral edema in those patients.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: The protocol presented in this study provides a new method for visualizing and assessing lymphatic flow in patients with HF. It allows for continuous, real-time tracking of lymphatic flow and enables examination of all lymphatic drainage pathways in the lower extremity, highlighting the importance of examining all lymphatic vessel pathways of the lower extremity, because the flow can differ between them. Overall, methods such as this can serve in propagating future research regarding the role of lymphatics in the pathophysiology of HF.

TRANSLATIONAL OUTLOOK: Although this method has some limitations, including the inability to perform serial flow assessments within a short period of time, it has several advantages and is safe and easy to perform. It could potentially help to understand the role of the lymphatic system in the pathophysiology of HF and, as a result, could aid in developing new ways of treatment for peripheral edema in those patients.

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