

Novel quantitative parameters of lymphatic contrast-enhanced ultrasound for breast cancer-related lymphoedema: Correlating with clinical staging

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ABSTRACT

Introduction: This study aimed to investigate the correlation between contrast-enhanced ultrasound (CEUS) characteristics of lymphatic vessels in affected limbs and International Society of Lymphology (ISL) clinical staging in patients with breast cancer-related lymphoedema (BCRL). Additionally, we sought to evaluate the validity of CEUS parameters in assessing lymphatic function.

Methods: Clinical and lymphatic CEUS data of 42 patients with BCRL were collected prospectively. The morphology of collecting lymphatic vessels (CLVs) was classified into five types: beaded, linear, tortuous-and-dilated, unevenly dilated, and blind-ended. The correlations between ISL clinical stages in BCRL patients and the following parameters were recorded: internal diameter of CLVs, the number of visualised CLVs, and the number of CLVs reaching above the elbow (CLV-RAE). Additionally, differences in these lymphatic parameters with different ISL stages were compared.

Results: A total of 346 CLVs were visualised in 42 BCRL patients, of which 246 CLVs were visualised above the elbow joint. The percentage of visualised CLV-RAE showed a stronger association with disease progression ($r = -0.74, p < 0.05$). The internal diameter of the thickest visualised CLVs demonstrated a more pronounced correlation ($r = 0.69, p < 0.05$). Morphological features of the CLVs showed significant staging correlations.

Conclusion: CEUS could accurately assess the structural and functional status of lymphatic vessels by quantitatively analysing critical parameters, including the number of CLVs, internal diameter, and imaging pattern.

Implications for practice: This study confirms the validity of CEUS parameters in assessing lymphatic vessel function and is expected to construct a quantitative assessment system for BCRL lymphatic function with the support of CEUS.

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Introduction

Lymphoedema was considered to be a chronic progressive disease, which was characterised by impaired lymphatic reflux. Moreover, lymphoedema was based on the dysfunction of the lymphatic vascular system caused by congenital or acquired factors, which could result in the abnormal accumulation of protein-rich lymphatic fluid in the interstitial space of tissues.^{1,2} Breast

cancer-related lymphoedema (BCRL) was referred to be a common type of secondary lymphoedema, and it tended to occur after the axillary lymph node destruction due to the surgical clearance or radiation therapy. Moreover, BCRL could manifest as a progressive increase in the swelling of the affected limb, alongside the induction of pathological changes including skin and subcutaneous tissue fibrosis, as well as the disruption of the structure of lymphatic vessels, thus resulting in the dysfunction of the limb activity.^{3,4} Notably, BCRL patients tended to be accompanied by impaired immune defence mechanisms, and the incidence of infectious complications (such as cellulitis) was significantly higher among these patients,⁵ which seriously affected their quality of life.

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Currently, the staging system proposed by the International Society of Lymphoedema (ISL) has been widely used for the clinical staging of lymphoedema,⁶ which provides an important reference for the selection of treatment options for patients.^{7–9} However, the manoeuvrability of lymphatic vessel tissue biopsy was relatively limited due to the high level of invasiveness and difficulty in the acquirement of samples. Therefore, there is an urgent need to develop an imaging technique that could dynamically and quantitatively assess the functional status of the lymphatic vessels, aiming to the accurate monitoring and assessment of changes in lymphoedema.

Contrast-enhanced ultrasound (CEUS) has been proven to have significant advantages in limb lymphatic vessel imaging due to its superior penetration, high spatial definition, and ability to clearly visualised collecting lymphatic vessels (CLVs).^{10,11} However, the technique remains in the preliminary stages of research, and a standardised evaluation system for lymphatic vessel ultrasonography is relatively underdeveloped. Our data show that the characteristic CEUS parameters (e.g., number of lymphatic vessels, internal diameter, type of visualisation, height of visualisation, etc.) of BCRL patients can be employed to assess lymphatic vessel function. Specifically, this study focused on the correlation between CEUS signs of lymphatic vessels in the affected limbs of BCRL patients and the clinical staging of ISL. Based on the above analysis, this study intends to discuss the validity of evaluating the CEUS parameters of lymphatic vessels to assess their function, and to provide a more reliable imaging basis for accurate diagnosis and the formulation of personalised treatment plans.

Methods

Patients

As a prospective exploratory study, a total of 42 consecutive BCRL patients who underwent lymphatic vessel ultrasonography in the Department of Ultrasound Medicine, The First Affiliated Hospital of Nanchang University between October 2022 and September 2024 were included in this study. All participants were female (57.0 ± 7.9 years) and exhibited unilateral upper limb lymphoedema following breast cancer surgery (Table 1). Approval was obtained from the University Ethics Committee, with the approval number IIT-2024-128. The sample size was determined based on the following considerations: ① The primary objective of this study was to identify feasible imaging parameters and

preliminarily select promising ones in CEUS, a novel method for assessing lymphatic vessel function. In line with previous pilot studies in lymphatic imaging,^{10,12} a sample size of 40–50 cases is widely accepted and sufficient for preliminary analyses. Future plans include larger-scale confirmatory studies based on these findings. ② Owing to constraints in surgical availability and extended follow-up requirements, 42 patients were ultimately included. This represents the complete set of high-quality data attainable under current conditions and is consistent with the sample sizes used in comparable pilot studies.^{13,14} It remains adequate for exploratory statistical purposes. Larger confirmatory studies are planned based on these findings.

Inclusion criteria: BCRL of the affected upper extremity occurred after breast cancer surgery; ISL stage 1 or higher⁶ (Table 2); Underwent ultrasonography of the lymphatic vessels and completed preoperative localisation one week prior to intra-operative exploration; Have been informed and signed an informed consent form approved by the Ethics Committee. Exclusion criteria: Have received standardised treatment for limb lymphoedema; In the period of cellulitis attack; With a history of other surgery or trauma to the affected upper limb; Comorbid lymphatic malformations, coeliac chest, or coeliac abdomen; Progression or recurrence of the primary disease; Allergy to CEUS contrast agent.

Instruments and methods

The study utilised a Siemens Acuson Sequoia ultrasound system equipped with contrast-enhanced functionality. To ensure adequate penetration depth while effectively controlling the mechanical index (MI) and avoiding premature microbubble destruction, a linear mid-frequency transducer (Model 10L4, 4–10 MHz) was employed, with the MI set between 0.06 and 0.08. The ultrasonic contrast agent used was SonoVue lyophilised powder (Bracco Company; sulphur hexafluoride microbubble content: 59 mg), which was reconstituted with 5 mL sterile saline to form a homogeneous suspension following the manufacturer-recommended agitation protocols. An experienced senior sonographer was employed to perform the CEUS examination on the affected limbs of the patients, and intradermal contrast injection was performed on the affected upper limbs between the finger webbing and at the ulnar-radial side of the palmar surface of the wrist joints. The dose of each injection point was designed as 0.5 mL, and the injection site was gently massaged locally for 5–15 s immediately after the injection to promote lymphatic vessel visualisation. The lymphatic vessels were real-time dynamic tracking along the long axis of the limb, with synchronous archiving of real-time imaging sequences. The following parameters were documented per case: the number of visualised CLVs, the type and internal diameter of CLVs, and the height of CLVs. Two senior and experienced sonographers interpreted the CEUS images of lymphatic vessels and diagnosed the classification of CLVs. In

Table 1
Patients' Characteristics.

Characteristics	Mean ± SD/n (%)
Age, years	57.0 ± 7.9
BMI, kg/m ²	24.1 ± 3.4
Gender	
Female	42 (100 %)
ISL stage	
ISL-1	4 (9.5 %)
ISL-2	26 (61.9 %)
ISL-3	12 (28.6 %)
Primary cause of disease	
Breast cancer	42 (100 %)
Upper limb lymphoedema	
Left	24 (57.1 %)
Right	18 (42.9 %)
Cellulitis	
No	25 (59.5 %)
Yes	17 (40.5 %)

SD, standard deviation; BMI, body mass index.
ISL, International Society of lymphoedema.

Table 2
Clinical criteria of staging lymphoedema in the consensus document of the international society of lymphology 2023.

Stage	Key Characteristics
0	Subclinical condition: Lymphatic impairment exists, but no visible or measurable swelling.
1	Swelling subsides with limb elevation or compression.
2	Limb elevation alone rarely reduces tissue swelling.
3	Lymphostatic elephantiasis and trophic skin changes: Skin thickening, hyperkeratosis, papillomas. Irreversible tissue damage and massive limb enlargement.

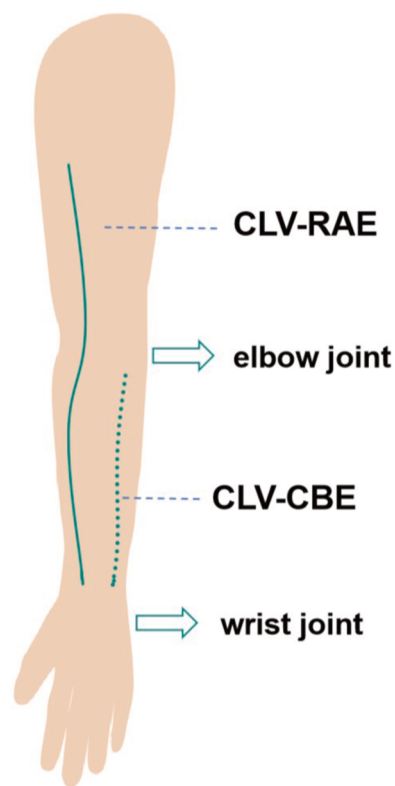


Figure 1. Schematic diagram of contrast-enhanced collecting lymphatic vessels in the palmar aspect of the upper limb. Following intradermal injection of contrast medium at the ulnar-radial side of the palmar surface, the lymphatic vessels were dynamically traced along the long axis of the limb. Green solid lines: Collecting lymphatic vessels reaching above the elbow joint (CLV-RAE). Green dashed lines: Collecting lymphatic vessels confined to below the elbow joint (CLV-CBE). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

case of diagnostic discrepancies, the diagnosis made by the more experienced sonographers was adopted. Two senior sonographers, blinded to all clinical information, independently assessed the images. These were presented in random order to prevent sequence bias, and each evaluator was unaware of the other's assessments. Intraoperative exploration for lymphatic-venous anastomosis (LVA) was performed within one week of CEUS completion for each patient.

CEUS performance

Number of visualised CLVs and visualised CLVs reaching above the elbow (CLV-RAE)

The number of the visualised CLVs among the patients was recorded, alongside the number of CLVs reaching above the elbow (CLV-RAE) (Fig. 1).

Characterisation and internal diameter of the CLVs

Type: According to the different internal diameters and patterns of visualised CLVs, the samples were classified into five types: beaded, linear, tortuous-and-dilated, unevenly dilated, and blind-ended (Fig. 2). Additionally, discontinuous bead-like distribution of contrast medium in CLVs was defined as the beaded type; a filled distribution of contrast medium in CLVs with spon- taneous lymphatic vessel alignment was defined as the linear

type; a filled distribution of contrast medium in CLVs with tortuous lymphatic vessel alignment was referred to as the tortuous-and-dilated type; a filled distribution of contrast medium in CLVs with uneven internal diameter was defined as the unevenly dilated type, and a filled or segmental distribution of contrast medium in CLV without visualisation of the near-centre was defined as the blind-ended type. Additionally, CLVs with combined features of uneven internal diameter and tortuous-and-dilated phenotypes would be classified as the uneven-diameter type (video 1).

Supplementary video related to this article can be found at doi:10.1016/j.radi.2025.103199

Statistical analysis

Data analysis was performed using SPSS 27.0 (IBM Corp). Normally distributed continuous variables were expressed as mean $\pm$ standard deviation (SD). The normality of data distribution was assessed by the Shapiro-Wilk test. For normally distributed data, one-way analysis of variance (ANOVA) was applied to compare intergroup differences. Non-normally distributed data were analysed using non-parametric tests (Kruskal-Wallis H test). If overall significance was detected ($p < 0.05$), post hoc pairwise comparisons were performed. Associations between CLVs visualisation types and ISL stages in BCRL patients were evaluated by Fisher's exact test, particularly for contingency tables with expected cell frequencies <5 . Spearman's rank correlation was used to assess relationships between: mean internal diameter of visualised CLVs, the internal diameter of the thickest CLVs, percentage of visualised collecting lymphatic vessels reaching above the elbow (CLV-RAE), and ISL stages in BCRL patients. The Kappa test was used to assess diagnostic consistency between two observers for CLV visualisation types. Statistical significance was defined as a two-tailed $p < 0.05$.

Results

Number of visualised CLV

A total of 346 CLVs were visualised in 42 patients, of which 24 CLVs were visualised in 4 patients with ISL-1 stage (24/346, 7.0 %), 242 CLVs in 26 patients with ISL-2 stage (242/346, 69.9 %), and 80 CLVs in 12 patients with ISL-3 stage (80/346, 23.1 %). Analysis indicated that the correlation between the number of visualised CLVs and the clinical stage of ISL was relatively weak ($p > 0.05$) (Table 3). However, more CLVs were visualised with the ISL-2 stage than the ISL-1 or ISL-3 stage, with a statistically significant difference ($p < 0.05$) (Fig. 3).

In this study, among the 346 CLVs, 246 (246/346, 71.1 %) of the CLVs were visualised above the elbow joint, including 24 (24/24, 100 %) in ISL-1, 190 (190/242, 78.5 %) in ISL-2, and 32 (32/80, 40.0 %) in ISL-3 (Fig. 3). A negative correlation was found between the percentage of CLV-RAE and ISL clinical stage in BCRL patients ($r = -0.74$, $p < 0.05$) (Fig. 4).

CLV visualisation type and internal diameter

Morphological analysis of CLVs indicated that the morphological characteristics of the 346 visualised CLVs exhibited significant staging correlation. Linear type CLVs accounted for the highest percentage (232/346, 67.1 %), followed by beaded type (59/346, 17.1 %) and tortuous-dilated type (45/346, 13.0 %), and unevenly dilated type (2/346, 0.6 %) and blind-ended type (8/346, 2.3 %) were relatively rare (Table 4). Linear lymphatics were predominant in ISL-1 and ISL-2 (91.7 % and 77.3 % of the total number in each

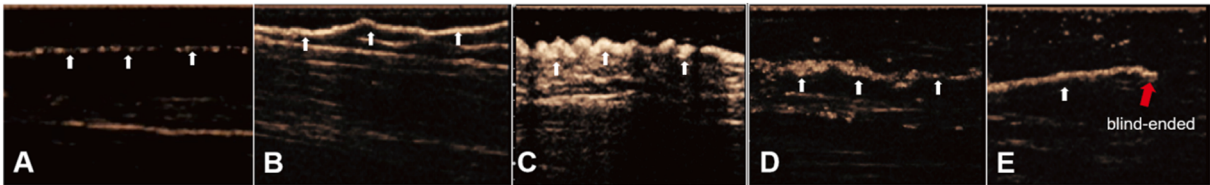


Figure 2. Five different types of collecting lymphatic vessels. A: beaded type (discontinuous bead-like distribution of contrast medium), B: linear type (uniform tubular structure with spontaneous lymphatic vessel alignment), C: tortuous dilatation type (tortuous lymphatic vessel alignment), D: uneven internal diameter type (uneven internal diameters along the vessel course), E: blind-ended type (no visualisation of the near-centre). White arrows point to visualised CLVs. Red arrows point to the blind ends of visualised CLV. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Parameters of visualised CLV across ISL stages in patients.

Variable	ISL-1	ISL-2	ISL-3	r (95 % CI)	p-value
Mean internal diameter of CLV (mm)	0.53 ± 0.05	0.84 ± 0.14 ^a	0.93 ± 0.25 ^a	0.44 (0.062, 0.745)	<0.05
Internal diameter of the thickest CLV (mm)	0.53 ± 0.05	0.90 ± 0.12 ^a	1.45 ± 0.42 ^{ab}	0.69 (0.425, 0.858)	<0.05
Number of the visualised CLV	6.00 ± 0.82	9.31 ± 1.62 ^a	6.67 ± 1.88 ^b	−0.23 (−0.585, −0.154)	0.12
Number of the visualised CLV-RAE	6.00 ± 0.82	7.31 ± 2.26	2.67 ± 1.97 ^{ab}	−0.55 (−0.733, −0.290)	<0.05
Percentage of visualised CLV-RAE (%)	100	77.68 ± 19.07	35.30 ± 24.66 ^{ab}	−0.74 (−0.849, −0.580)	<0.05

^a vs. ISL-1 group, $p < 0.05$.
^b vs. ISL-2 group, $p < 0.05$. r = Spearman's rank correlation coefficient. CLV = collecting lymphatic vessel. ISL=International Society of Lymphology. CLV-RAE = collecting lymphatic vessels reaching above the elbow joint.

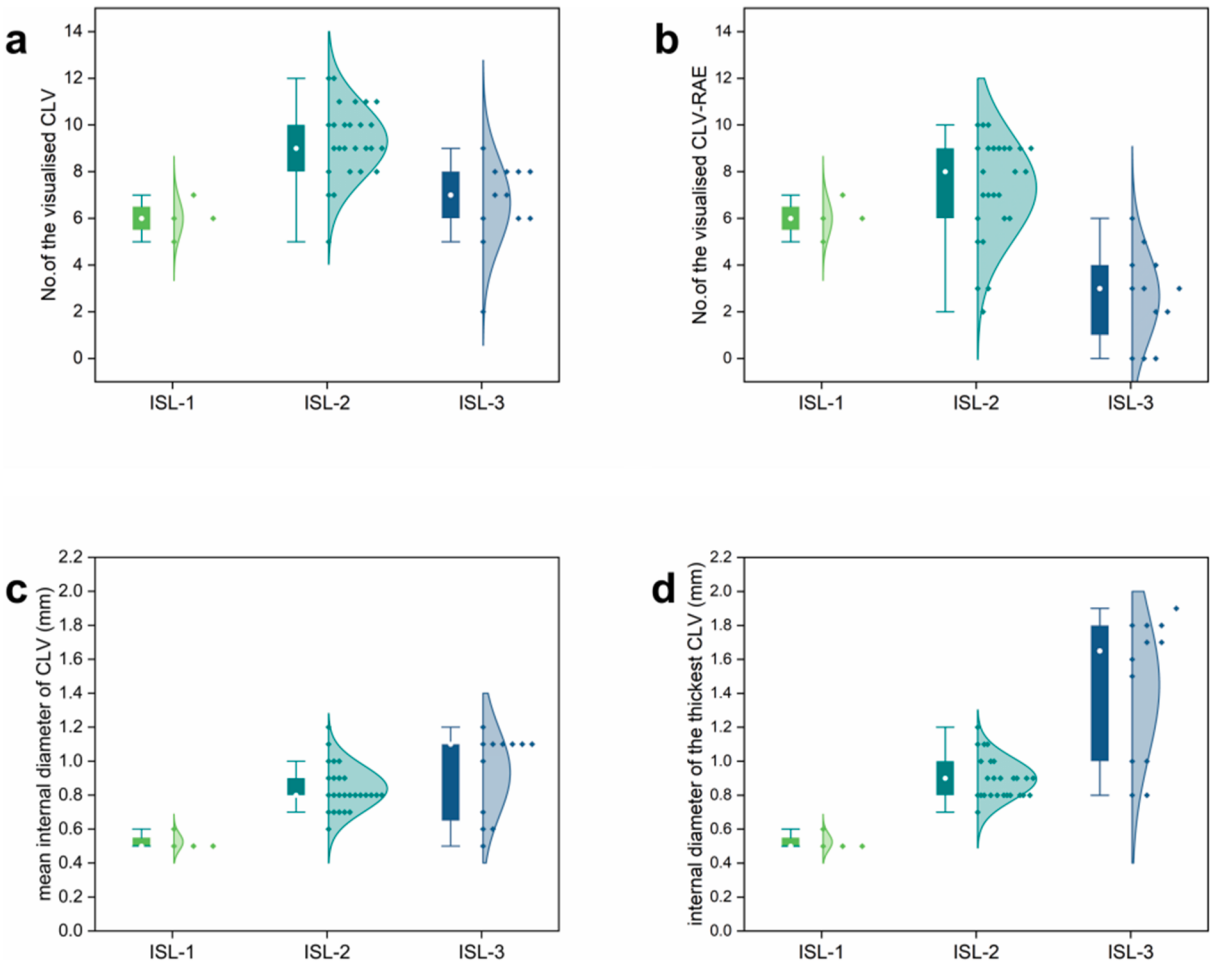


Figure 3. Half-box half-violin plots demonstrating the opacification height and internal diameter of collecting lymphatic vessels (CLVs) across International Society of Lymphology (ISL) stages.(a) Distribution of the number of visualised CLVs in patients stratified by ISL stage.(b) Distribution of CLVs with visualised height above the elbow joint across ISL stages.(c) Distribution of the mean internal diameter of visualised CLVs by ISL stage.(d) Distribution of the internal diameter of the thickest CLVs by ISL stage.

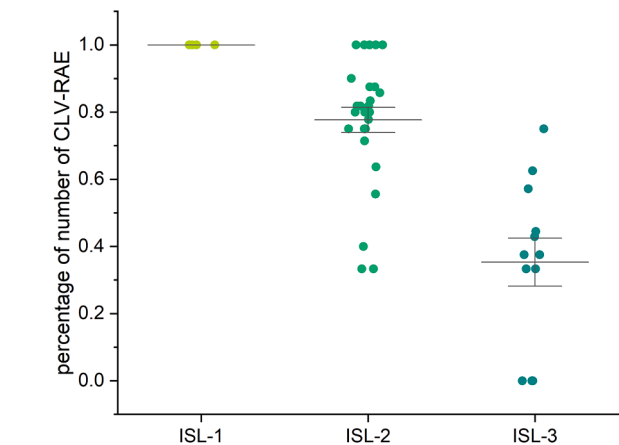


Figure 4. Bar-scatter plot comparing the percentage of visualised collecting lymphatic vessels reaching above the elbow joint (CLV-RAE) between International Society of Lymphology (ISL) stages. Scatter dots indicate individual patients (n = 42).

stage, respectively), whereas beaded type showed a progressive increasing trend in ISL-2 (11.6 %) and ISL-3 (36.3 %). Tortuous-dilated and blind-ended types were concentrated in ISL-3, accounting for 27.5 % and 5.0 % of lymphatics in this stage, respectively. Additionally, their incidence was significantly higher than that in stage ISL-1 (32.5 % vs 0) and ISL-2 (32.5 % vs 11.2 %), with a statistically significant difference between groups ($p < 0.05$). Furthermore, the unevenly dilated phenotypes were only observed in the ISL-3 stage (Fig. 5).

The results revealed that the mean internal diameter of CLVs was 0.53 ± 0.05 mm in ISL-1 patients, 0.84 ± 0.14 mm in ISL-2 patients, and 0.93 ± 0.25 mm in ISL-3 patients. Additionally, Spearman's correlation analysis indicated a positive correlation between the mean internal diameter of CLVs and clinical lymphoedema stage ($r = 0.44$, $p < 0.05$). Remarkably, the internal diameter of the thickest CLVs detected by CEUS exhibited a stronger correlation with ISL stage ($r = 0.69$, $p < 0.05$) (Table 3).

Consistency analysis: Two senior sonographers independently diagnosed the classification of 346 CLVs, and good consistency was observed between the two sonographers ($\kappa = 0.87$) (Table 5).

Discussion

With the continuous increase in the incidence of breast cancer globally, secondary lymphoedema caused by BCRL has become a major clinical concern in the field of clinical diagnosis and treatment.⁶ Although individualised treatment strategies can be developed according to the stage of lymphoedema, the lack of standardised lymphatic function assessment systems has resulted in insufficient objective criteria for clinical decision-making. Currently, traditional lymphatic vessel visualisation techniques are mainly composed of indocyanine green (ICG) fluorescent

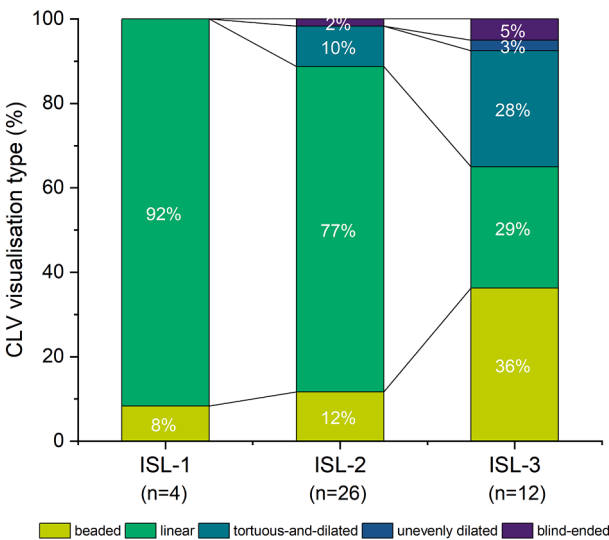


Figure 5. Collecting lymphatic vessels (CLV) visualisation type. Percentage stacked bar chart: Proportions of different opacification types in CLVs across International Society of Lymphology (ISL) stages. Based on imaging characteristics, the visualised CLVs were categorized into five morphological types: beaded type, linear type, tortuous dilatation type, uneven internal diameter type, blind-ended type.

lymphography, lymphoscintigraphy, and magnetic resonance lymphangiography (MRL).^{7,15–17} Previous studies revealed that the combination of ICG with dynamic fluorescence imaging and lymphatic vessel nuclide imaging can be used for the pathological staging of lymphoedema by observing the dermal backflow (DBF) patterns.^{14,18} Additionally, MRL can be used to grade the lymphatic vessels by analysing the DBF and the extent of the CLVs.¹⁶ However, the efficacy of the existing imaging techniques in the visualisation of the deep CLVs remains to be clarified. Moreover, there are some significant limitations that restrict the imaging efficacy of existing imaging techniques for deep collecting lymphatics: ICG fluorescent lymphography is difficult to display deep structures due to its insufficient depth of tissue penetration (<1.5 cm), and MRL exhibits low sensitivity for the dynamic assessment of lymphatic reflux.^{19,20} This results in the anatomical-functional matching bias in the staging of lymphoedema, which negatively affecting the accuracy of therapeutic plans.

In recent years, CEUS has demonstrated unique advantages in dynamic visualisation of CLVs.^{11,21} As demonstrated, intradermal administration is an effective technique.^{22,23} The dermis contains an extensive network of initial lymphatic capillaries with wide endothelial gaps, which readily absorbs large molecules such as microbubble contrast agents. The agent is subsequently transported centripetally via lymphatic drainage into collecting lymphatic vessels, enabling their visualisation.²⁴ According to our preliminary work, CEUS could accurately locate the anatomical alignment of the CLVs, and it could realise the functional assessment for the affected limbs of BCRL patients based on the internal diameters of CLVs, the type of imaging, and other dynamic

Table 4
Classification of CLV across ISL stages in patients.

Stage	beaded	linear	tortuous-and-dilated	unevenly dilated	blind-ended	Total	<i>p</i>
ISL-1 (n = 4)	2 (8.3 %)	22 (91.7 %)	0 (%)	0	0	24	
ISL-2 (n = 26)	28 (11.6 %)	187 (77.3 %)	23 (9.5 %)	0	4 (1.7 %)	242	
ISL-3 (n = 12)	29 (36.3 %)	23 (28.7 %)	22 (27.5 %)	2 (2.5 %)	4 (5.0 %)	80	
Total (n = 42)	59	232	45	2	8	346	<0.001

n = number of BCRL patients; The percentages represent the proportion of each lymphangiographic pattern among the visualised lymphatic vessels in patients at the same ISL stage.
CLV = collecting lymphatic vessel. ISL=International Society of Lymphology.

Table 5
Consistency test of CLVs typing by two sonographers

sonographer2	sonographer1					Total
	beaded	linear	tortuous-and-dilated	unevenly dilated	blind-ended	
Beaded	55	19	0	0	0	74
Linear	4	213	0	0	0	217
Tortuous-and-dilated	0	0	44	0	0	44
Unevenly dilated	0	0	1	2	0	3
Blind-ended	0	0	0	0	0	8
Total	59	232	45	2	8	346

parameters in the lymphatic system.^{21,25} However, the lack of a standardised assessment system in the current quantitative analysis of lymphatic vessel ultrasonography remains a significant concern. Due to this technical bottleneck, the correlation between characteristic CEUS parameters (e.g., number of CLVs, type of visualisation, height of visualisation, etc.) and the clinical staging of ISL in patients with BCRL has been considered an important research direction, and it is crucial for the optimisation of the staging of lymphoedema and developing individualised treatment plans.

Sheng et al. suggested that lymph vessels exhibit further dilation and larger diameters in the subcutis of the affected forearm with lymphoedema progression.²⁶ Notably, the internal diameters of the CLVs tended to dilate progressively with advancing clinical lymphoedema stages (Fig. 3), but the mean internal diameter showed no statistically significant difference between the stage of ISL-2 and the stage of ISL-3 ($p > 0.05$). Morphological characterisation showed that the predominantly beaded (30/266, 11.3 %) and linear (209/266, 78.6 %) CLVs were visualised in ISL-1 and ISL-2, and the tortuous-and-dilated types (23/242, 9.5 %) occurred in ISL-2 (Table 4). The maximum internal diameter of the CLVs in ISL-3 patients (1.45 ± 0.42 mm) was significantly higher than that of ISL-1 (0.53 ± 0.05 mm) and ISL-2 (0.90 ± 0.12 mm), which might

be attributed to the presence of tortuous dilatation type with uneven dilatation predominantly in ISL-3 patients. However, our results indicated that the number of CEUS visualised CLV strips showed limited correlation with the ISL stage, although patients with ISL-2 showed more visualised strips versus those with ISL-1 and ISL-3 ($p < 0.05$). Additionally, two characteristic changes were observed in CLVs of ISL-3 patients: 1) tortuous-and-dilated CLVs were present in 8 patients (8/12,66.7 %), with 2 of these patients (2/8, 25.0 %) exhibiting uneven internal diameter or blind ends, and 2) bead-type CLVs were also predominant in ISL-3 patients. These phenomena might be attributed to structural remodelling of the lymphatic vessel wall: with disease progression, a combination of smooth muscle cell proliferation and collagen fibre deposition in the wall resulted in a significant decrease of the wall elasticity, alongside the narrowing and sclerosis of the lumen.²⁷ This bi-directional pathology could explain the “paradox” of the increase in maximum internal diameter and the decrease in the number of visualised strips in ISL-3 patients. Moreover, sclerotic lymphatic vessels in some ISL-3 patients were difficult to visualise due to the luminal narrowing or occlusion, whereas the dilated segments were dilated from reduced wall elasticity. This further contributed to the formation of a characteristic pattern of tortuous-and-dilated and beaded coexistence. Simultaneously, an unevenly dilated phenotype could be observed in some of the internal diameters of the CLVs.

Based on the dynamic tracking of the CEUS imaging pattern, a negative correlation was also found between the percentage of CLV-RAE and the progression of lymphoedema ($r = -0.74$, $p < 0.05$). The height of CLV visualisation exhibited a progressively decreasing trend with the escalation of the ISL stage: all the patients (24/24, 100 %) with the stage of ISL-1 exhibited the height of CLVs visualised above the elbow joint, whereas the patients with ISL-2 stage had 190 (190/242, 78.5 %) CLVs visualised above the elbow joint. Regarding the 12 patients with ISL stage 3, 25 % of them were limited to the height below the elbow joint. This phenomenon could be attributed to the fact that the wall elasticity of the lymphatic vessels exhibited a gradually decreasing trend with

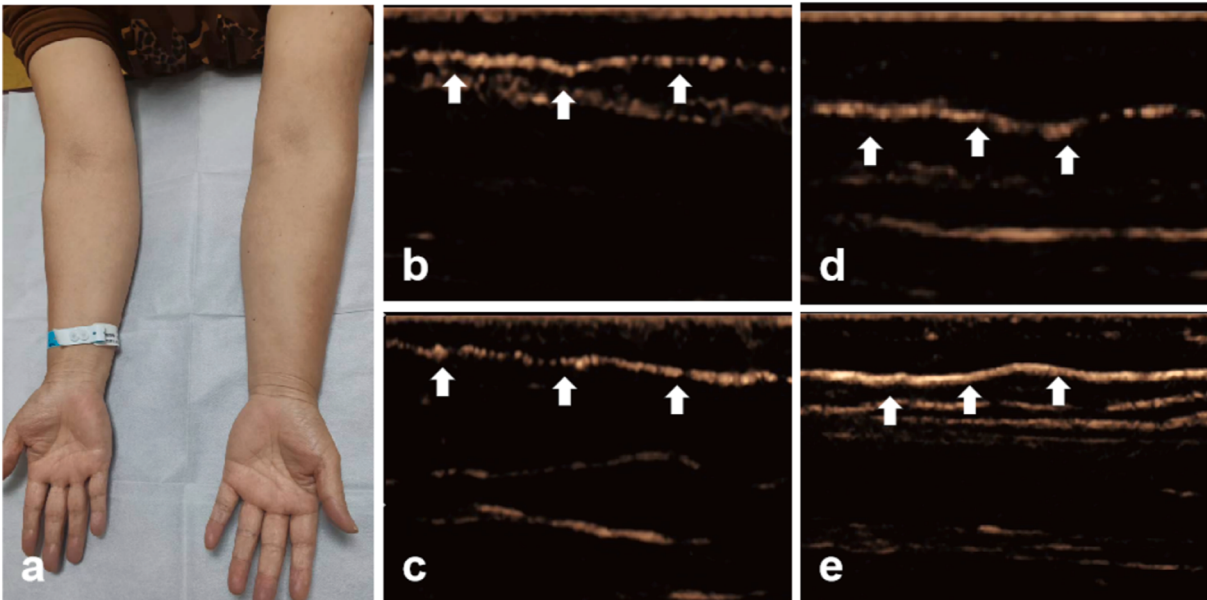


Figure 6. A 60-year-old female presented with secondary left upper limb lymphoedema 12 months after modified radical mastectomy and axillary lymph node dissection for breast cancer. The patient reported a history of cellulitis in the affected limb one month prior to the current evaluation. In the affected limb, four CLVs were visualised. Among these, three exhibited a linear type, and one displayed a beaded type. The opacification of all CLVs was confined to below the elbow joint. Panels b–d show the beaded CLVs, while panel e demonstrates the linear ones. White arrows point to visualised CLVs.

the progression of the disease. The contractile function was progressively weakened, alongside the increasing difficulty in the upward mobility of the lymphatic fluid. Besides, the lymphatic fluid retrograde flow caused by lymphatic valve insufficiency served as one of the reasons.^{28,29} In addition, it might be related to the significant decrease in forearm lymphatic fluid transit rate after the treatment of breast cancer.³⁰ According to the previous MRL studies,¹⁶ the imaging of CLV tended to deteriorate with the disease stage, which was consistent with the results of this study. The contrast agent of 3 patients with ISL-3 stage was almost sloughed off at the point of injection, and only 1–2 CLVs were visualised and the height of visualisation was limited to below the elbow joint. According to the intraoperative exploration, the lymphatic vessel functions of these patients were extremely poor, which was consistent with the ultrasound findings. Therefore, LVA surgery was not performed. Based on the above findings, this study suggests that LVA surgery should not be performed immediately in patients with a small number of visualised CLVs and visualisation height limited to below the elbow joint.

In this study, we identified an ISL stage 2 patient with severely compromised lymphatic function (Fig. 6), and only 4 CLVs were visualised by CEUS, of which 3 were in the form of beads. Additionally, the height of visualisation within all the CLV was limited below the elbow joint, and the intraoperative exploration found that the internal diameter of the CLVs was extremely narrow and the vessel walls were inflexible. The question about the clinical history revealed that the patient had a short postoperative period of 12 months and a history of cellulitis one month prior to the research, which might result in the inconsistency between the poor lymphatic vessel function and the staging of the lymphoedema. Therefore, for patients with a recent history of cellulitis and poor lymphatic vessel function indicated by lymphatic CEUS, it is recommended that standardised anti-inflammatory treatment be administered first. Once the inflammation is stably controlled, re-evaluation should be performed to determine their suitability for LVA surgery.

Based on these findings, surgeons may refer to the CEUS characteristics proposed in this study—such as number of visualised CLVs, visualisation type, internal diameter—to more accurately select functional collecting lymphatics for anastomosis. This approach could potentially reduce the risk of surgical failure and improve the success rate of LVA. Furthermore, CEUS may help identify patients with severely impaired lymphatic function who are currently unsuitable for LVA. For these individuals, unnecessary surgery could be avoided in favour of prioritising conservative physical therapy or anti-inflammatory treatment.

However, this study had some limitations: ① As a single-centre exploratory prospective study with a small sample size; ② The injection method for CEUS has not yet formed a unified standard. As a non-conventional approach, only intradermal injection was used in this study, which may introduce potential bias and affect the generalisability of the findings. ③ There is a lack of comparative analysis of CEUS data on lymphatic vessels in patients prior to breast cancer surgery; ④ Lack of a gold standard reference: A key limitation of this study is the absence of histopathological confirmation to rigorously validate the diagnostic accuracy of individual ultrasound features. ⑤ This study did not include a control group of healthy lymphatic vessels evaluated by CEUS, nor did it investigate the correlation with long-term outcomes.

Conclusion

As an effective method of lymphangiography, CEUS is able to clearly visualise the type of CLVs and the thickest inner diameter, and the percentage of CLV-RAE exhibited a significant connection

with the ISL staging. It could be concluded that the above parameters could be employed in the functional assessment of lymphatic vessels in CEUS, providing the anatomical basis for its quantitative assessment. In addition, CEUS could promote the development in the clinic due to its advantages of non-irradiation, convenient operation, and high spatial resolution.³¹ Therefore, CEUS is expected to exhibit crucial functions in the field of lymphoedema diagnosis and treatment, promoting the development of precise diagnosis and treatment of lymphoedema.

Ethics approval and consent to participate

Ethical approval for this study was obtained from Medical Ethics Committee of the First Affiliated Hospital of Nanchang University and Clinical Medicine Ethics Review China (Approval number: IIT-2024-128).

Written informed consent was obtained for anonymised patient information to be published in this article.

Animal Welfare: Not applicable. No animals were used in this research.

Availability of data

Data required for this study may be made available by the author(s) upon reasonable request.

Author contributions

Z.J.L: Data curation, Writing- Original draft preparation, Investigation, Methodology, Visualization.

Z.S.Z: Data curation, Validation, .

P.F: Investigation, Resources.

J.L.W: Visualization, Resources.

J.Z: Visualization.

Z.R.L: Resources.

L.L.Z: Conceptualization, Methodology, Writing - Review & Editing, Supervision, Funding acquisition, Project administration.

Generative AI use

Not applicable.

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Conflict of interest statement

None.

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