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BREAST IMAGING

ORIGINAL ARTICLE

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Ultrasound-guided marker placement and blue dye staining for sentinel lymph node biopsy in early-stage breast cancer

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PURPOSE

Ultrasound (US)-guided marker placement is an emerging method for sentinel lymph node biopsy (SLNB), and this study aimed to investigate the concordance between US-guided marker placement for peri-foraminal lymph nodes (LNs) in the axillary cribriform fascia and blue dye-stained SLNs, especially the utility of the two concurrent labeling methods for SLNB in patients with early-stage breast cancer.

METHODS

Female patients with clinically staged T1-2N0M0 breast cancer were enrolled. Patients received US-guided marker placement on the day before surgery, followed by blue dye staining. SLN biopsy was performed on the blue dye-stained LNs first and then on the US-guided markers.

RESULTS

A total of 96 eligible patients were enrolled. The detection rates of US-guided localization, blue dye, and their combination were 100.0%, 95.8%, and 100.0%, respectively. Among the 92 patients with blue dye staining, US-guided markers were found in blue-stained LNs from 91 patients (concordant) but failed in 1 patient (non-concordant); among the 4 patients without blue-stained LNs, US-guided markers were found in all 4 patients (non-concordant), resulting in a general concordance rate of 94.8% (91/96). Notably, among the 92 patients with blue dye staining, 23 had LN metastasis diagnosed through SLNB, whereas among the 4 patients without blue-stained LNs, the SLNB was performed based on US-guided markers, which revealed 3 patients with LN metastasis and 1 without. Moreover, combining the two labeling methods concurrently identified 26 patients with LN metastasis.

CONCLUSION

US-guided localization combined with blue dye concurrently may be a satisfied option for SLNB detection.

CLINICAL SIGNIFICANCE

US-guided localization combined with blue dye offers an accessible, anatomically grounded, and radiation-free strategy for optimizing the SLNB procedure, especially in settings where radioactive isotopes are not available.

KEYWORDS

Breast cancer, ultrasound-guided marker placement, axillary cribriform fascia, blue dye, sentinel lymph node biopsy

Breast cancer remains the most prevalent cancer in women, especially those aged between 40 and 49 years, with approximately 2.31 million new cases and 0.67 million deaths every year worldwide, seriously threatening women's physical and mental health.[1,2] Surgical treatment is a fundamental component of the comprehensive management of breast cancer, with axillary lymph node dissection (ALND) enabling accurate LN staging, removing potential metastatic LNs, achieving superior local recurrence control, and improving

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the survival of patients.[3-5] However, ALND is associated with several complications, such as upper lymphedema, shoulder dysfunction, paresthesia, brachial plexus injury, and wound infection, which seriously affect patients' recovery and quality of life.[6-8]

With improvements in breast cancer screening, an increasing number of patients with breast cancer are being diagnosed with clinically negative axillary LNs at an early stage.[9,10] In this context, the clinical utility of sentinel LN biopsy (SLNB) has become prominent, as the first node or group of nodes receiving lymphatic drainage from the primary tumor can be used to estimate the pathological status of all axillary LNs and serve as a key predictor of recurrence risk and overall survival in patients with breast cancer.[11,12] Due to its high accuracy, the status of the SLN is critical for decisions regarding both the type of surgery and related treatments.[7,11] Moreover, SLNB is associated with less trauma and fewer complications and is widely applied in patients with early-stage breast cancer with clinically negative axillary LNs to avoid unnecessary ALND.[11,13]

SLN tracing methods mainly include the blue dye method (such as methylene blue, patent blue, and isosulfan blue), the nuclide method combined with the dye method, and the fluorescence tracer method.[14-16] The nuclide method (e.g., ^{99m}Tc) offers high detection rates but with radiation exposure, logistical complexity, and limited accessibility in many institutions.[15] The fluorescence method (e.g., indocyanine green) provides real-time visualization but requires specialized equipment and has limited tissue penetration.[16] By contrast, the blue dye method is simple, intuitive, and widely available, yet its detection rate (approximately 91%–95%) and false-negative rate remain suboptimal.[17] These comparative limitations contextualize the rationale for developing an accessible, radiation-free adjunct, such as ultrasound (US)-guided marker placement, to complement the blue dye and improve SLNB accuracy, particularly in resource-limited settings where nuclide and fluorescence techniques are unavailable.[15-17] However, the blue dye method still has the disadvantages of a relatively low detection rate and high false-negative rate; therefore, the exploration of better methods for SLN tracing and subsequent SLNB remains vital.[18]

Main points

- Ultrasound (US)-guided marker placement demonstrated high concordance with blue dye for sentinel lymph node biopsy (SLNB) in breast cancer.
- US-guided marker placement combined with blue dye achieved a satisfied detection rate of SLNB in breast cancer.
- Collectively, US-guided localization plus blue dye may be an accessible, anatomically grounded, and radiation-free strategy for optimizing SLNB in breast cancer.

US-guided marker placement is a technique that is being increasingly explored for the precise localization of breast lesions and LNs. It facilitates accurate surgical excision and allows for the effective tracking of tumor response throughout neoadjuvant chemotherapy.[19,20] Notably, US-guided marker placement is simple, stable, and safe for axillary LNs in patients with breast cancer.[21] Moreover, our previous study revealed that SLNB via the axillary cribriform fascia (ACF) with blue dye anatomically covering the SLN area improved intraoperative blood loss, drainage flow, and upper limb dysfunction.[22] The ACF is formed by the fusion of superficial and deep fascial layers, creating natural foramina that serve as obligate passageways for breast lymphatic drainage into the axilla; consequently, LNs situated adjacent to these foramina represent the first echelon of nodal filtering, functionally defining them as SLNs.[23,24] This anatomical framework provides the logical basis for US-guided targeting of peri-foraminal LNs as surrogates for true SLNs. Thus, we hypothesized that the US-guided identification of peri-foraminal LNs in the ACF may represent SLNs, but related studies are rare.

This study aimed to investigate whether US-guided marker placement of peri-foraminal LNs in the ACF is concordant with blue dye-stained SLNs and to determine whether combining these two methods improves SLNB performance in patients with early-stage breast cancer.

Methods

Patients

From May 2023 to July 2024, female patients with clinically staged T1-2N0M0 invasive breast cancer who were scheduled to undergo SLNB were prospectively enrolled from the Department of Breast Surgery, Tangshan People's Hospital, China. The inclusion criteria were as follows: (1) aged ≥ 18 years; (2) unifocal, primary breast cancer confirmed by core needle biopsy; and (3) no clinical or imaging evidence of axillary LN metastasis from US. Patients with prior axillary surgery, neoadjuvant chemotherapy, or systemic metastases were excluded. This study was approved by the Ethics Committee of Tangshan People's Hospital with approval number RMYY-LLKS-2024023 and approval date 5/24/2024. Moreover, this study was registered on the Chinese Clinical Trial Registry (https://www.chictr.org.cn) with the access number ChiCTR2400087357. In addition, all patients provided written informed consent.

Ultrasound-guided marker placement

US examination was performed using a Philips EPIQ 7 ultrasound system (Philips Healthcare, Bothell, WA, USA) equipped with a high-resolution linear transducer (eL18-4 MHz). US-guided marker placement in peri-foraminal LNs in the ACF was performed using an UltraClip™ breast tissue marker needle with a US-enhanced coil (Bard, USA). In brief, on the day prior to surgery, the patients underwent high-resolution US examination of the axilla. Attention was given to the cribriform foramen area adjacent to the mammary gland in the affected axilla, particularly the ACF, cribriform foramen, and LNs; then, the LN closest to the mammary gland and nearest to the ACF foramen was identified (Supplementary Figure 1a-f). A sterile 18G marker needle was subsequently inserted into the cortical margin of the identified LN under real-time US guidance, carefully avoiding the adjacent vascular structures (Supplementary Figure 2a-f). The needle position and corresponding US images were documented, and the skin overlying the insertion site was marked to guide intraoperative identification.

Blue dye

On the day of surgery, 2–3 mL of 1% methylene blue dye was injected subcutaneously into the periareolar region and around the tumor of the affected breast approximately 15 minutes before the skin incision.

Sentinel lymph node biopsy procedure

A standard SLNB procedure was performed through a small axillary incision based on the blue dye and US-guided markers. First, blue dye-stained LNs were carefully identified and excised (Supplementary Figure 3a-f). Second, the surgeon explored the region corresponding to the US-guided marker placement, and all visible LNs (not only the marker-localized ones) within this anatomically defined area were retrieved, regardless of the presence of dye staining.

Third, visual observation and radiographic examination were performed that confirmed the presence of the US-guided marker in the excised LN specimens (Supplementary Figure 4a-d). Finally, all excised LNs were subjected to intraoperative frozen section analysis and routine histopathological examination.

Sample size calculation

The minimum sample size was calculated on the basis of the following settings: a paired design, a blue dye detection rate estimated at 90%, a US-guided marker placement detection rate estimated at 100%, power of 90%, and α: 0.05 (two tailed), resulting in a minimum sample size of 89 cases. This study included 96 patients, which exceeded the minimum sample size.

Data collection and outcome measures

Patient characteristics, including age, tumor size, clinical TNM stage, tumor location, and molecular subtypes, were recorded. The primary outcome was the concordance rate between US-marked LNs in the ACF area and blue dye-stained SLNs. The secondary outcomes included the number of detected LNs, number of detected cases, detection rate (by cases), average number of LNs per case, and SLNB pathological findings. Detection rate (%): (number of detected cases/all cases) × 100%.

Statistical analysis

For statistical analysis, SPSS version 26.0 (IBM Corp., Armonk, NY, USA) was used. Data are presented as the mean ± standard deviation, n, %, or n (%). Comparisons were performed using the chi-square test, Fisher's exact test, Student's t test, or McNemar test, as appropriate. A two-sided *P* value < 0.05 was considered statistically significant.

Results

Study flow

A total of 100 patients with breast cancer were initially screened, with 2 excluded because they had axillary LN metastasis, 1 because of prior axillary surgery, and 1 because of neoadjuvant therapy. The remaining 96 patients were eligible and included in this study. The patients underwent US-guided localization and marker placement of peri-foraminal LNs in ACF on the day before surgery. On the day of surgery, blue dye staining was performed, and SLNB was conducted first on the blue dye-stained LNs and then on the US-guided markers (Figure 1).

Patient characteristics

The mean age of the included patients was 41.4 ± 7.9 years, with a mean tumor size of 1.3 ± 0.3 cm (Table 1). Thirty-nine (40.6%) and 57 (59.4%) patients had clinical TNM stage I and IIA disease, respectively. Fifty-nine (61.5%), 13 (13.5%), 14 (14.6%), and 10 (10.4%) patients had tumors located in the upper outer quadrant, lower outer quadrant, upper inner quadrant, and lower inner quadrant, respectively. Moreover, 30 (31.2%), 33 (34.4%), 21 (21.9%), and 12 (12.5%) patients were classified as luminal A, luminal B, human epidermal growth factor receptor 2 positive, and triple negative, respectively.

Comparison of detection rates

A total of 293 LNs in 96 patients were detected by US-guided localization, with a detection rate of 100.0%, whereas a total of 282 LNs in 92 patients were detected by blue dye staining, resulting in a detection rate of 95.8% (Table 2). Therefore, the detection rate was not significantly different between US-guided localization and blue dye staining ($P = 0.125$). Furthermore, the average number of LNs detected per patient was 3.05 ± 0.49 by US-guided localization and 3.07 ± 0.46 by blue dye staining, with no significant difference between the two methods ($P = 0.851$). Notably, when the two labeling methods (blue dye + US) were combined, the detection rate reached 100.0%, with an average number of LNs detected per case of 3.06 ± 0.52.

Concordance between ultrasound-guided localization and blue dye staining

In the 92 patients with blue dye staining, US-guided markers were found in blue dye-stained LNs from 91 patients (concordant) but failed in 1 patient (non-concordant). In the 4 patients without blue dye staining, US-guided markers were found in all 4 (non-concordant). Taken together, the concordance rate between US-guided localization and blue dye staining was 94.8% (Table 3).

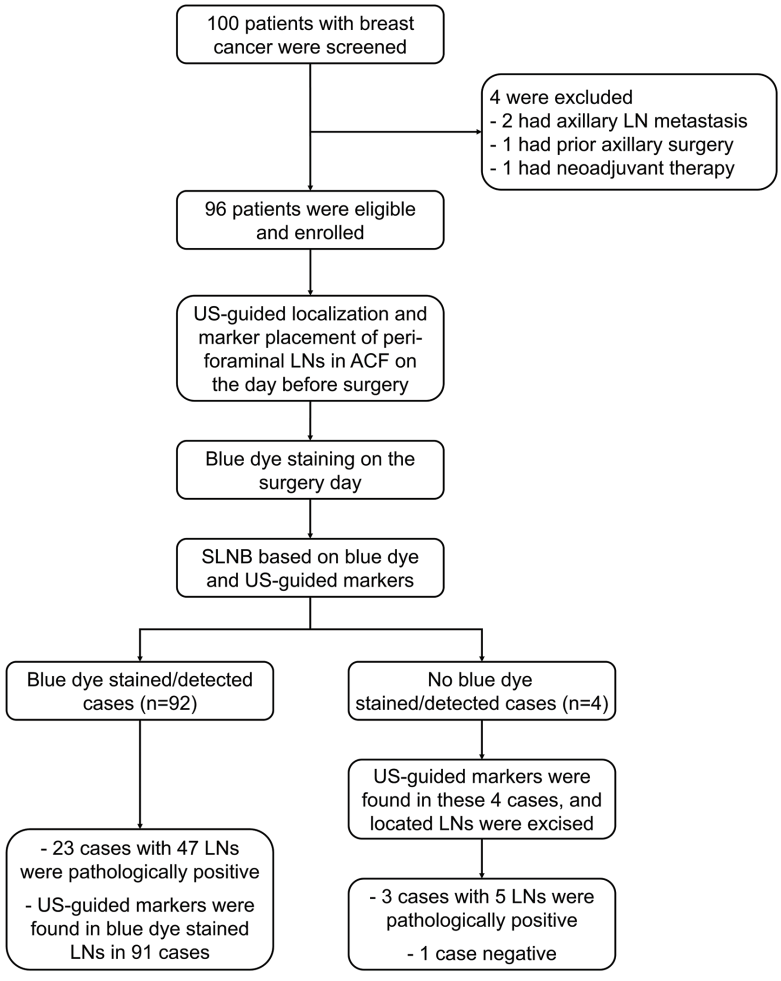


Figure 1. Study flowchart illustrating patient enrollment and procedures. SLNB, sentinel lymph node biopsy; US, ultrasound; LN, lymph node; ACF, axillary cribriform fascia.

Pathological findings from sentinel lymph node biopsy

In the 92 patients with blue dye staining, 23 had positive LN metastasis revealed by SLNB, consisting of 47 positive LNs. Notably, in the 4 patients with no blue dye staining, a sum of 11 LNs were excised based on the sites of the US-guided markers and proposed for biopsy, revealing 3 patients with positive LN metastasis (consisting of 5 positive LNs) and 1 patient with negative LN metastasis. This indicated that US-guided localization improved the accuracy of SLNB. Moreover, as a result of LN identification using the blue dye combined with US-guided localization method, 26 patients were pathologically confirmed through SLNB to have positive LN metastasis, resulting in the identification of 52 positive LNs (Table 4). In addition, among the 91 concordant cases, 22 patients had positive LN metastasis (consisting of 45 positive LNs).

Safety profile of ultrasound-guided marker placement

In general, no adverse events or complications directly related to the US-guided marker placement procedure were observed. Moreover, no marker dislodgement, migration beyond the LN capsule, or infections were observed.

Table 1. Patient characteristics

Parameters	Patients with breast cancer (n = 96)
Age (years), mean ± SD	41.4 ± 7.9
Tumor size (cm), mean ± SD	1.3 ± 0.3
Clinical TNM stage, n (%)	
I	39 (40.6)
IIA	57 (59.4)
Tumor location, n (%)	
Upper outer quadrant	59 (61.5)
Lower outer quadrant	13 (13.5)
Upper inner quadrant	14 (14.6)
Lower inner quadrant	10 (10.4)
Molecular subtype, n (%)	
Luminal A	30 (31.2)
Luminal B	33 (34.4)
HER2 positive	21 (21.9)
Triple negative	12 (12.5)

SD, standard deviation; TNM, tumor-node, and metastasis; HER2, human epidermal growth factor receptor 2.

Table 2. Lymph node detection using ultrasound and blue dye

Parameters	Number of detected LNs	Number of detected cases	Detection rate by cases	P_1 value	Average LNs per case	P_2 value
US (n = 96)	293	96	100.0%	0.125	3.05 ± 0.49	0.851
Blue dye (n = 96)	282	92	95.8%		3.07 ± 0.46	
Blue dye + US	294	96	100.0%	–	3.06 ± 0.52	–

P_1 represented the comparison of detection rates between US and blue dye; P_2 represented the comparison of average LNs per case between US and blue dye. LN, lymph node; US, ultrasound.

Table 3. Concordance rate between ultrasound and blue dye detection

Parameters		In blue dye-detected cases		Concordance rate
		Yes (n = 92)	No (n = 4)	
Ultrasound detection	Concordant	91	0	94.8%
	Non-concordant	1	4	

Table 4. Pathological findings from SLNB

Parameters	SLNB positivity by LNs	SLNB positivity by cases
Blue dye detected (92 cases)	47	23
Blue dye non-detected but US detected (4 cases)	5	3
US detected (96 cases)	50	25
Blue dye + US detected (96 cases)	52	26

SLNB, sentinel lymph node biopsy; US, ultrasound; LN, lymph node.

Discussion

Currently, the need for ALND in patients with early-stage breast cancer with negative axillary LNs remains a subject of debate.[7,25,26] Previous studies have indicated that patients with negative SLNs may be exempt from ALND, suggesting that SLNB can effectively serve as a substitute for ALND for early-stage breast cancer.[26-28] Moreover, SLNB has been established as a safe, straightforward, and reliable technique that helps patients avoid a range of complications associated with ALND.[11]

A comprehensive meta-analysis revealed that blue dye is a standard labeling method for SLNB, with a detection rate of approximately 91% (95% CI: 88%–94%) in patients with breast cancer.[17] Moreover, combining it with another labeling method concurrently further improved the detection rate of blue dye for SLNB.[29,30] For instance, indocyanine green and blue dye concurrently achieve a detection rate of 98.5%,[29] and Tc99-labeled sulfur colloid plus blue dye concurrently achieves a detection rate of 96.4%–100.0%[30] for SLNB in patients with breast cancer. US-guided localization has also been applied to SLNB in breast cancer, with a detection rate of 96.3%.[31] However, investigations that combine US-guided marker placement and blue dye methods lack sufficient evidence. In the present study, US-guided localization, blue dye-guided staining, and their combination achieved detection rates of 100.0%, 95.8%, and 100.0%, respectively, for SLNB in patients with early-stage breast cancer, indicating that US-guided localization improves the detection rate of blue dye.

Importantly, this study focused on assessing the concordance between US-guided localization of peri-foraminal LNs in the ACF and traditional blue dye-stained SLNs in patients with early-stage breast cancer and revealed an exceptionally high concordance rate of 94.8%, indicating that the peri-foraminal LNs in the ACF visualized under US are highly representative of true SLNs. This strong overlap supports the anatomical reliability of US-guided localization based on axillary fascial structures. Anatomically, the peri-foraminal region in the ACF corresponds to a critical convergence area for breast lymphatic drainage. The ability of US to visualize these structures and target LNs within or adjacent to the foramen provides a solid anatomical basis for its use in SLN identification.[32,33]

Although the concordance was high, one patient exhibited "skip metastasis" in this study, where the metastatic SLN lay outside the US-visible peri-foraminal region in the ACF. Although rare, such conditions have been reported previously.[34,35] Skip metastasis, which is commonly recognized as the metastatic involvement of higher-level axillary nodes without level I nodes, occurs in approximately 1.5%–5.3% of breast cancer cases.[36] Within the ACF-based anatomical framework, several mechanisms may explain this phenomenon. First, accessory lymphatic channels may bypass the peri-foraminal region entirely, traversing the ACF by non-sieve routes.[33,37] Second, tumor-induced lymphatic obstruction can reroute flow into collateral pathways that circumvent typical first-echelon nodes.[38] Third, interindividual variations in the number and distribution of cribriform foramina may result in incomplete lymphatic capture by a single peri-foraminal node. Thus, although US-guided marker placement is highly accurate, it should be complemented by intraoperative exploration, particularly in anatomically variant cases.

In this study, among the 92 patients with blue dye staining, 23 were confirmed by SLNB to have LN metastasis; notably, among the 4 patients who did not have blue dye staining, LNs were excised on the basis of US-stained sites, after which 3 patients were confirmed by SLNB to have LN metastasis and 1 was negative. These findings suggest that US can reliably identify metastatic SLNs when the blue dye method fails, particularly in patients who are elderly or obese, thereby reducing the risk of false negatives and supporting more precise surgical decision-making.[39]

Beyond clinical performance, the cost profile of US-guided marker placement combined with blue dye warrants consideration, particularly when compared with alternative dual-tracing methods for SLNB. The most widely established dual-tracing approach in many high-resource settings is the combination of radioactive colloid (e.g., ^{99m}Tc-labeled sulfur colloid) with blue dye. However, this method incurs substantial costs related to nuclear medicine facilities, radiopharmaceutical preparation, gamma probe maintenance, and personnel training for radiation handling and waste disposal.[40,41] In resource-limited or non-specialized centers where such infrastructure is unavailable, the isotope-based dual tracing may not be feasible. Another increasingly used dual modality is indocyanine green combined with blue dye, which eliminates radiation but requires a near-infrared fluorescence imaging system and dedicated cameras, representing a substantial capital investment and recurrent maintenance cost.[42,43] Regarding the US-guided marker placement, its cost mainly lies in a standard high-resolution US machine (commonly already present in the institution) and a disposable marker needle, whose combination with blue dye was less costly than radioactive colloid combined with blue dye and indocyanine green combined with blue dye. Specifically, the cost of US-guided marker placement combined with blue dye is approximately Chinese Yuan (CNY) 600 in our institution, whereas that of radioactive colloid combined with blue dye is approximately CNY 1,200 and that of indocyanine green combined with blue dye is approximately CNY 1,400.

Despite the clinical and cost advantages discussed above, it is important to acknowledge a disadvantage—US-guided marker placement requires a pre-operative procedure with US clipping on the day before surgery. This entails an additional hospital visit, a second needle insertion under US guidance, and the placement of one or more metallic markers into axillary LNs.

Several limitations of this study should be mentioned. First, as a single-center study with a moderate sample size, the generalizability of the study findings is limited, and future multicenter studies with larger cohorts are warranted. Second, the superficial projection of LNs relative to the ACF may vary with changes in patient positioning between US localization and surgery, introducing potential bias. Third, this study did not employ subcutaneous contrast-enhanced US for LN imaging, which could improve the precision of identifying the primary draining SLN. Fourth, ALND was not performed to clarify the false-negative rate of US-guided localization.

In conclusion, US-guided localization of peri-foraminal LNs in the ACF exhibits a satisfactory detection rate and has high concordance with blue dye-stained SLNs in patients with early-stage breast cancer. Notably, US-guided localization combined with blue dye offers an accessible, anatomically grounded, and radiation-free strategy for optimizing SLNB, particularly in settings where radioactive isotopes are not available. However, further validation with large-scale samples and multicenter studies is still needed.

Footnotes

Conflict of interest disclosure

The authors declared no conflicts of interest.

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Supplementary Figures 1-4: https://d2v96fxpocvxx.cloudfront.net/72b4fafb-5db4-4d61-bedf-c8100360bb15/content-images/eeb5ea0d-1253-4fa5-b4bf-decf87486bf2.pdf

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