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Review

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Advantages of contrast-enhanced ultrasound in the localization and diagnostics of sentinel lymph nodes in breast cancer

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Abstract: Sentinel lymph nodes (SLNs) are the first station of lymph nodes that extend from the breast tumor to the axillary lymphatic drainage. The pathological status of these LNs can predict that of the entire axillary lymph node. Therefore, the accurate identification of SLNs is necessary for sentinel lymph node biopsy (SLNB) to replace axillary lymph node dissection (ALND). The quality of life and prognosis of breast cancer patients are related to proper surgical treatment after the precise identification of SLNs. Some of the SLN tracers that have been identified include radioisotope, nano-carbon, indocyanine green (ICG), and methylene blue (MB). However, these tracers have certain limitations, such as pigmentation, radiation dangers, and the requirement for costly detection equipment. Ultrasound contrast agents (UCAs) have good specificity and sensitivity, and thus can compensate for some shortcomings of the mentioned tracers. This technique is also being applied to SLNB in patients with breast cancer, and can even provide an initial judgment on SLN status. Contrast-enhanced ultrasound (CEUS) has the advantages of high distinguishability, simple operation, no radiation harm, low cost, and accurate localization; therefore, it is expected to replace the traditional biopsy methods. In addition, it can significantly enhance the accuracy of SLN localization and shorten the operation time.

Key words: Breast cancer; Sentinel lymph node (SLN); Contrast-enhanced ultrasound (CEUS); Ultrasound contrast agent (UCA)

1 Introduction

According to the International Agency for Research on Cancer (IARC) on the latest global cancer data for 2020, breast cancer has officially surpassed lung cancer as the most common cancer in the world, seriously endangering the health and survival of women (Chen et al., 2017; Zaheer et al., 2019; Du et al., 2022; Yu et al., 2022). There are many types of breast cancer, but the surgical methods are basically the same (Vtorushin et al., 2022). Tumor resection and function preservation are two major considerations for breast surgeons during surgery. Sentinel lymph nodes (SLNs) are the first station or group of lymph nodes for the primary tumor to develop into lymphatic metastasis. Sentinel lymph node biopsy (SLNB) has been widely used in clinics to best preserve the upper limb function of patients and reduce the recurrence of cancer, preventing many patients from the loss of upper limb function caused by excessive axillary dissection (Qiu et al., 2018). If there is no metastasis in the SLN, it is basically certain that the downstream lymph nodes are also free from metastasis. Thus, axillary lymph node dissection (ALND) can be avoided (Fig. 1). The accurate identification of SLNs is a prerequisite for SLNB to replace ALND. In the search for SLNs, ultrasound contrast agents (UCAs) are anticipated to be a new generation of tracers because of their rapid metabolism in the body, low toxicity, lack of radiation, ability to visualize lymphatic vessels and lymph nodes in real time, as

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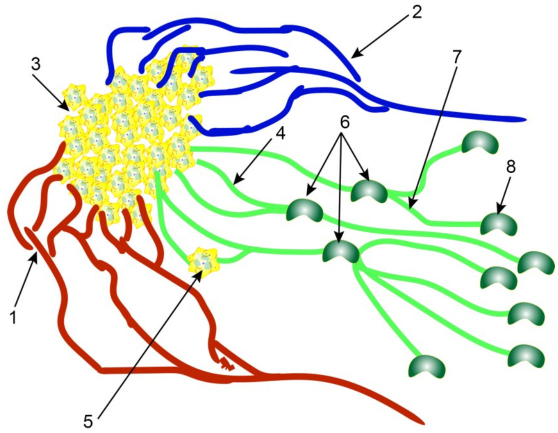

Fig. 1 Lymphatic drainage for breast cancer. 1: Arteries; 2: Veins; 3: Primary breast tumor; 4: Sentinel lymphatic channel (SLC); 5: Breast tumor cell; 6: Sentinel lymph nodes (SLNs); 7: Downstream lymphatic channel; 8: Downstream lymph node.

well as their ability to assist in judging the benignity and malignancy of tumors or lymph nodes.

2 Significance of effective SLN identification

With our deepening knowledge of breast cancer and the improving accuracy of tumor surgery, the resection range of breast cancer surgery is gradually narrowing. In patients with definite axillary lymph node metastasis, ALND should be administered without delay, while in those with ambiguous axillary lymph node metastasis, SLNB should be performed first. If there is no metastasis in the SLN and the probability of axillary metastasis is very low, ALND can be avoided and upper limb function can be preserved as much as possible (Magnoni et al., 2020). However, approximately 60% of early breast cancer patients without axillary lymph node metastasis also receive ALND according to some reports (Parks and Cheung, 2017). Furthermore, common ALND complications, such as upper limb numbness, shoulder pain, upper limb dyskinesia, poor lymphatic reflux leading to upper limb edema, upper limb deep venous thrombosis, and others (Monleon et al., 2016; Ballal et al., 2018; Belmonte et al., 2018), have a significant negative impact on patients' postoperative quality of life. Therefore, the correct evaluation of axillary lymph node status can maximize the protection of breast cancer patients from the deleterious effects of disease progression. Additionally, it serves as an important basis for determining the prognosis and directing treatment for breast cancer. SLNB can be performed in the following cases: (1) early invasive breast cancer; (2) clinical axillary lymph node enlargement but a negative result of puncture pathology; (3) single or multicentric lesions in the primary breast; (4) in situ cancer undergoing mastectomy; (5) negative axillary lymph nodes before and after neoadjuvant therapy (Gherghe et al., 2015; Garcia-Etienne et al., 2020).

3 Tracers used to find SLNs

The main SLN tracers employed at present are methylene blue (MB), nano-carbon, indocyanine green (ICG), radioisotope, superparamagnetic iron oxide, mitoxantrone hydrochloride, etc. Firstly, MB is the most commonly used one in the clinic in China, whereas excessive dosage or injection time will make non-SLNs stained, increase the number of lymph nodes resected, and lead to more complications caused by surgery. On the other hand, MB can result in long-term pigmentation at the injection site and partial skin necrosis in severe cases (Fig. 2). Furthermore, the small size of MB particles makes them diffuse widely throughout the breast tissue, thereby targeting the SLNs inaccurately (Peek et al., 2017; Cwalinski et al., 2020). Secondly, due to the short staining duration and the low intensity for the lymphatic channel, nano-carbon performs poorly in distinguishing SLNs from other non-SLNs (Tjo and Varamini, 2022). Thirdly, the most frequent adverse impact of radionuclides is radiation hazard. It also has the disadvantage of a high rate of missed detections (Krynyckyi et al.,

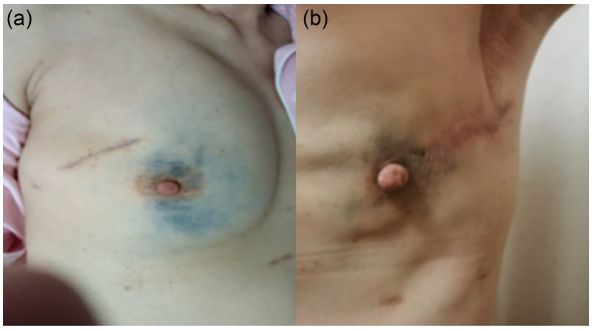

Fig. 2 Pigmentation due to methylene blue (MB). (a) Skin pigmentation after breast conservation surgery. (b) Hyperpigmentation after total mastectomy with preserved nipples.

2000). Fourthly, although the locations of lymphatic arteries and nodes can be observed more clearly with ICG, it is not widely utilized in clinical practice due to its high cost, long processing time, and short fluorescence penetration (Kitai and Kawashima, 2012; Grischke et al., 2015; Majlesara et al., 2017). Superparamagnetic iron oxide relies on changes in the local magnetic field, with hand-held magnetometer employed to instantly locate SLNs during operation. Besides, it provides the benefits of an extended injection window and simplicity in preparation. Disappointingly, it is primarily employed abroad and has no evidence-based support in China (Zheng et al., 2018). Mitoxantrone hydrochloride is a novel biological self-assembled nanocrystal originally developed in China, which is able to drain through the lymphatic vessels and enrich the regional lymph nodes. Despite its theoretically good targeting ability and visibility, we found that it could cause skin redness, swelling, and induration (Yang et al., 2021). The comparisons were listed in Table 1.

4 Contrast-enhanced ultrasound in breast cancer

The above-mentioned tracers all have limitations, so researchers have focused on developing UCAs that have the advantages of fast metabolism, minimal toxic side effects, low incidence of allergies, and ability of the rapid (15–30 s) real-time observation of the imaging process (Ntoulia et al., 2021).

4.1 Contrast-enhanced ultrasound

Contrast-enhanced ultrasound (CEUS) refers to the injection of UCA into a vein or local tissue where it flows along lymphatic or blood vessels. Similar to the role of the contrast medium in computed tomography (CT) and magnetic resonance imaging (MRI), the tissue containing the UCA can increase the contrast with the surrounding tissue in ultrasound imaging, and it can also be tracked and tagged (Saidha et al., 2018). Omoto et al. (2006) first used CEUS to visualize SLNs in breast cancer patients. The results demonstrated that CEUS with 25% albumin could be used to locate SLNs. Subsequently, UCAs progressed from the initial 25% albumin to a new generation of microbubble contrast agents such as SonoVue and Sonazoid. Many studies have demonstrated the superior development effect of Sonazoid compared to SonoVue (Cui et al., 2013; Zhai et al., 2019). CEUS was applied in the early diagnosis of benign and malignant liver diseases, which could be judged by the action of the enhanced images. As indicated above, the utilization of CEUS technology has gradually grown to cover various auxiliary diagnoses of benign and malignant disorders, such as those of the urinary system, reproductive system, gastrointestinal system, endocrine system, and neurological malignancies (Cantisani et al., 2015; Kearns et al., 2019; Sorrenti et al., 2021).

4.2 Identification and diagnosis of SLNs

Routine ultrasound examination is the most common method for the preoperative evaluation of the axillary lymph node status. Despite the high detection rate for suspicious axillary lymph nodes, the localization and imaging accuracy of lymph nodes are generally poor in patients with or without micro-metastasis of the axillary lymph node. Besides, conventional grayscale ultrasound cannot determine whether a lymph node is an SLN, while CEUS can display lymphatic vessels in real time and identify SLNs at the first lymph node

Table 1 Deficiencies of different contrast agents

Contrast agent	Deficiency	References
MB	1. Excessive removal of LNs 2. Pigmentation 3. Skin necrosis 4. Low targeting specificity	Peek et al., 2017; Cwalinski et al., 2020
Nano-carbon	Low targeting specificity	Tjo and Varamini, 2022
ICG	1. High cost 2. High time requirement	Kitai and Kawashima, 2012; Grischke et al., 2015; Majlesara et al., 2017
Radioisotope	Radiation hazard	Krynyckyi et al., 2000
Superparamagnetic iron oxide	Lack of evidence-based basis	Zheng et al., 2018
Mitoxantrone hydrochloride	1. Skin induration 2. High cost	Yang et al., 2021

MB: methylene blue; ICG: indocyanine green; LNs: lymph nodes.

enhancement. Moreover, it can be used to judge the benign and malignant by SLN enhancement in many patients with axillary lymph node negatively diagnosed by conventional ultrasound, reducing the false negative rate.

Moody et al. (2017) used QUADAS-2 to assess the quality of the research approach by searching several electronic databases. Calculations were made to determine the sensitivity and specificity of identifying lymph node metastasis. The recognition rate of SLNs by CEUS was 70%–100%, and the sensitivity and specificity for SLNs metastasis detection were 54% and 100%, respectively. It was concluded that CEUS can replace conventional tracers such as isotopes and blue dyes for the identification and localization of SLNs. By collecting some kinds of published literature and conference reports, Cui et al. (2020) determined the joint risk ratio of tumor metastasis between SLNs differentiated by CEUS (CE-SLNs) and SLNs not identified by CEUS (non-CE-SLNs). Moreover, they evaluated the accuracy of CE-SLNs in the combined diagnosis of the pathological states of all SLNs. The findings demonstrated that, in patients with early breast cancer, the sensitivity and specificity of SLN metastases were 98% and 100%, respectively, and that the identification rate of CE-SLNs was 70%–100%. Therefore, preoperative CEUS was regarded to improve the accuracy of locating SLNs (Cui et al., 2020). Through a meta-analysis of 16 related articles published on PubMed before September 2020, Huang SY et al. (2021) found that the sensitivity and specificity of CEUS to SLNs were 88% and 90%, respectively, and the area under the curve (AUC) was 0.9405, indicating that the CEUS has high sensitivity and specificity and can accurately diagnose the metastatic state of SLNs. Wang LN et al. (2021) collected clinicopathological data from 224 patients treated with SLNB. Logistic regression analysis was implemented to establish the risk prediction model of SLN transfer. Based on the β value of each variable in the model, the SLN metastasis risk scoring system was established and validated by the internal population. The AUC was 0.8766, and the sensitivity and specificity were 68.8% and 94.7%, respectively. This strongly suggests that CEUS can be used as a key indicator to assess SLN metastasis prior to surgery. Wang XJ et al. (2021) compared two methods of identifying SLNs: MB-coupled CEUS and straightforward MB staining. The combination of CEUS and MB had a higher recognition rate than MB alone. In addition, there was no discernible difference in recurrence-free survival (RFS) between the two groups. In the clinical trial conducted by Hu et al. (2020), 3D-CEUS was an effective tracer for detecting the location and metastatic status of SLNs, with a detection rate, sensitivity, and specificity of 95.3%, 75.0%, and 93.0%, respectively. The detailed above parameters were listed in Table 2.

Table 2 Role of contrast-enhanced ultrasound (CEUS) in the recognition of sentinel lymph nodes (SLNs) in breast cancer

Reference	Recognition rate (%)	Sensitivity (%)	Specificity (%)
Moody et al., 2017	70.0–100.0	54.0	100.0
Cui et al., 2020	70.0–100.0	98.0	100.0
Huang SY et al., 2021		88.0	90.0
Wang LN et al., 2021		68.8	94.7
Hu et al., 2020	95.3	75.0	93.0

4.3 SLN enhancement mode under CEUS

Under the action of UCA, the enhancement of SLNs can be classified into three types: type I, homogeneous enhancement; type II, non-homogeneous enhancement; and type III, no enhancement (Fig. 3). Zhao et al. (2018) discovered that, of the 124 SLNs shown by CEUS, all lymph nodes with type I enhancement pattern were found to be non-metastatic SLNs (51/51), 62% of type II lymph nodes were non-metastatic (47/76), and all type III enhancement pattern SLNs were metastatic (7/7). Deriving the enhancement pattern of CEUS can help to identify metastatic SLNs. Similar conclusions were obtained by Xie et al. (2015). Niu et al. (2023) further subdivided the non-uniform enhancement into focal defect enhancement and non-uniform enhancement, which further improved the accuracy of the classification.

Lymphatic tissue and lymphatic sinus make up the majority of lymph nodes, which comprise numerous lymphoid nodules. From the input duct, lymph enters the lymph sinus and leaves through the output duct. SLNs can be formed by the convergence of a single lymphatic vessel or by the convergence of two or more lymphatic vessels. When tumor cells invade the lymphatic vessels or lymph nodes, they can be completely or partially blocked, which prevents UCA from flowing in and causes no enhancement or partial

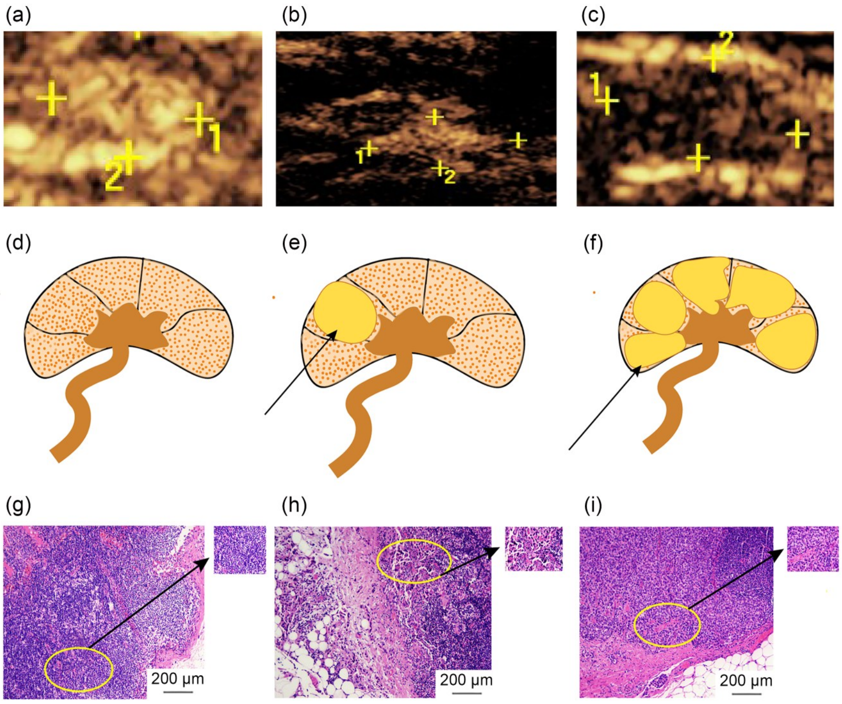

Fig. 3 Lymph node enhancement pattern under contrast-enhanced ultrasound (CEUS). (a) Type I: homogeneous enhancement; (b) Type II: non-homogeneous enhancement; (c) Type III: no enhancement. (d, e, f) The pattern diagrams of the three enhancement types of sentinel lymph nodes (SLNs), respectively, and the arrow is the unenhanced area. (g, h, i) Histological images of the three enhancement types of SLNs, respectively, where the arrow indicates an enlarged view of the relevant tissue.

enhancement (Mattrey et al., 2002; Goldberg et al., 2004; Luo et al., 2022). Regarding the benign lymph nodes, partial non-enhancement may be brought on by the smooth muscle contraction of lymphatic veins, which causes lymphatic fluid to reflux (MacDonald et al., 2008).

4.4 CEUS-related parameters

The time-intensity curve (TIC) of CEUS reflects the flowing speed and quantity of UCA in the tumor microvasculature. TIC analysis is a state-of-the-art CEUS video quantification technique that incorporates various quantitative parameters of blood perfusion. The quantitative parameters of blood perfusion mainly include peak intensity (PI, the maximum intensity of the TIC during the push), time to peak (TTP, time from zero intensity to peak), rise time (RT, the difference between TTP and the time of the first microbubble reaching the lesion), mean passage time (MTT), wash-in slope (WIS, the ratio of PI to TTP), rise slope (AS, the slope of the rising branch of TIC, showing the average perfusion rate after the presence of contrast agent and reflecting the local tissue perfusion rate), and AUC. Each of the above parameters can be used for qualitative assessment of the benign or malignant nature of primary breast masses or lymph nodes (Toki et al., 2021). TIC analysis in breast cancer patients typically shows shortened RT and TTP, as well as increased PI and AS (Lee YJ et al., 2019; Wang J et al., 2021; Guo et al., 2022).

4.5 Roles of CEUS in primary breast tumors

Nowadays, ultrasound and mammography comprise routine examinations in clinics to screen for breast tumors. However, their sensitivity and specificity are low for borderline tumors, leading to delayed treatment or over-treatment. Aspiration pathology is the gold standard, but it is an invasive procedure (Kemp Jacobsen et al., 2015). Numerous researches conducted in recent years have concluded that CEUS is of high value in discriminating between benign and malignant breast tumors (Saracco et al., 2012; Yuan et al., 2013).

4.5.1 Role of CEUS in the diagnosis of breast tumors

Compared with static breast ultrasound images, CEUS can provide more detailed information on tumor blood supply, bringing a more detailed description of the tumor (Zhou et al., 2020). Ioannidis et al. (2022) used the exponentially modified Gaussian (EMG) and gamma variate function (GVF) to analyze 25 patients with high suspicion of breast cancer. By combining CEUS data, such as wash-in, wash-out, TTP, and time to the maximal slope (TMSP), the analysis revealed the high diagnostic performance of EMG, with a sensitivity and specificity of (89.2±10.7)% and (70.0±18.5)%, respectively, which could effectively distinguish benign and malignant of breast masses (Ioannidis et al., 2022). Consistently, Chen et al. (2021) presented a predictive model for the diagnosis of breast cancer based on the features of CEUS in the training set of breast cancer patients, and determined its sensitivity as 97.2% and accuracy as 86.3% on the validation set, which were higher than those of the normal ultrasound. Furthermore, Zhou et al. (2020) carried out a meta-analysis of various articles on the ability of CEUS to distinguish benign from malignant breast masses published before April 2019, and found that the overall sensitivity and specificity of CEUS in distinguishing benign and malignant breast masses were 88% and 82%, respectively. This further confirmed the excellent performance of breast CEUS in differentiating benign and malignant breast lesions. Further analysis revealed that the TTP and WIS parameter values of CEUS were able to differentiate between granulomatous lobular mastitis (GLM) and breast cancer. Yin et al. (2022) retrospectively analyzed the quantitative and qualitative parameters of CEUS imaging for GLM and breast cancer, and found that the sensitivity and specificity of TTP in the diagnosis of GLM were 73.33% and 84.48%, respectively. Meanwhile, the sensitivity and specificity of WIS in the diagnosis of GLM were 86.21% and 70.00%, respectively (Yin et al., 2022). CEUS image features and parameters can also distinguish different subtypes of breast cancer, such as TTP (the shortest parameter in HER-2-positive breast cancer) and PI (the shortest in Luminal A breast cancer) (Wen et al., 2022).

4.5.2 Role of CEUS in neoadjuvant chemotherapy for breast cancer

Neoadjuvant chemotherapy (NAC) is the standard treatment for patients with locally advanced breast cancer. It is used to decrease tumor sizes and has the potential to operate on surgically inoperable patients. The treatment and prognosis of patients significantly depend on an early and accurate assessment of the effectiveness of NAC. Nevertheless, due to the heterogeneity of breast cancer, it is challenging to assess the tumor response to NAC. For instance, there might not be a discernible decrease in tumor size in responding tumors. Furthermore, tumor fragmentation brought on by treatment may influence the assessment of tumor efficacy. Huang YX et al. (2021) discovered that the AUC could be raised to 0.841 in the multivariate model combining CEUS parameters and molecular subtypes, and its sensitivity and specificity were 0.786 and 0.745, respectively, which could precisely determine the curative effect of the tumor on NAC. What is more, breast tumors that have undergone NAC frequently exhibit heterogeneous signal enhancement under CEUS, with the predominant enhancement pattern being “fast in and slow out.” Even breast tumors with high malignancy might show the “sun sign” under CEUS, which denotes a poor prognosis for patients (Gu et al., 2019). These mentioned parameters were listed in Table 3.

Table 3 Roles of contrast-enhanced ultrasound (CEUS) in the identification of breast tumors

Reference	Sensitivity (%)	Specificity (%)
Ioannidis et al., 2022	89.2	70.0
Chen et al., 2021	97.2	86.3
Zhou et al., 2020	88.0	82.0
Yin et al., 2022	TTP: 73.3 WIS: 86.2	TTP: 84.4 WIS: 70.0
Huang YX et al., 2021	78.6	74.5

TTP: time to peak; WIS: wash-in slope.

The aforementioned studies demonstrated that CEUS has a high recognition rate for SLNs and exhibits high sensitivity and specificity in the diagnosis of benign and malignant SLNs or breast masses. Meanwhile, a few large samples and multicenter prospective clinical trials remain that require further investigation.

5 Injection methods of UCAs

UCAs can be injected via a variety of techniques to localize SLNs. At present, subcutaneous or intradermal injection via the areola and peritumor injection is the most widely employed technique. The reference information of Sections 5.1–5.3 was listed in Table 4.

Table 4 Effects of different injection methods on sentinel lymph nodes (SLNs)

Reference	Method	Recognition rate (%)	Sensitivity (%)	Specificity (%)
Li et al., 2019	Intradermal	98.20	96.82	91.91
Hao et al., 2021	Intradermal	100.00	92.31	84.21
Xie et al., 2015	Intradermal	97.10	81.80	86.20
Li et al., 2022	Intradermal	96.37	86.47	89.81
Hu et al., 2020	Intradermal	75.00	93.00	95.30
Liu et al., 2019	Subcutaneous	71.17	98.04	49.23
Luo et al., 2021	Intradermal, subcutaneous	95.64		
Machado et al., 2023a	Peri-tumor	88.50		100.00
Machado et al., 2023b	Peri-tumor	53.00		53.00

5.1 Intradermal areola injection

Li et al. (2019) analyzed the clinical records of 453 patients with early breast cancer who had undergone SLNB. The standard control was blue dye. It was found that the recognition rate, sensitivity, and specificity of SLNs by the intradermal injection of UCAs around the areola were 98.20%, 96.82%, and 91.91%, respectively. This suggests that UCAs could be a useful alternative to blue dye. Importantly, Hao et al. (2021) even discovered that after the intradermal injection of UCAs, the recognition rate of SLN was higher than that of MB (95.59%), the sensitivity to SLNs was 92.31%, and the specificity was 84.21%. According to the clinical study on CEUS conducted by Xie et al. (2015), the recognition rate of SLNs by percutaneous CEUS was 97.10%, which is higher than that of the blue dye method. The sensitivity and specificity of the enhanced mode for recognizing the metastatic state of SLNs were 81.80% and 86.20%, respectively. In line with the earlier findings, these data indicate that percutaneous CEUS has considerable clinical application value. Additionally, 92 patients with early invasive breast cancer from six tertiary-class hospitals in China participated in a large-scale multicenter clinical trial on the localization and diagnosis of SLNs by CEUS, which was launched by Li et al. (2022) in China. The relevant data were collected and analyzed by the intradermal injection of UCAs through the areola. The results indicated that the recognition rate of SLNs by CEUS was 96.37%. Stratified analysis revealed that the detection rate of SLNs in patients with body mass index (BMI)<26 was as high as 99.25%. When used to diagnose SLN metastases, CEUS had sensitivity and specificity of 86.47% and 89.81%, respectively. Thus, CEUS is a feasible and useful method for the preoperative localization of SLNs, and it helps to diagnose SLN metastasis (Li et al., 2022).

5.2 Subcutaneous areola injection

Liu et al. (2019) retrospectively analyzed 75 patients with early breast cancer confirmed by pathology. CEUS was performed after the subcutaneous injection of UCA (SonoVue, total dose 2.0 mL) around the areola of the affected breast. The enhanced lymphatic vessels and SLNs were observed and tracked in real time, while they were drawn and labeled on the surface of the skin. SLNB was carried out after 2.0 mL MB was administered into the same injection site as SonoVue. Compared with the blue dye injection technique, the detection rate of CEUS in SLNs was 71.17%, and the sensitivity and specificity were 98.04% and 49.23%, respectively. Consistently, Luo et al. (2021) found that the recognition rate of SLNs by intradermal combined with subcutaneous injection of UCAs was 95.64%, higher than that of MB (92.05%), which could be used as a new method for SLNB.

5.3 Peri-tumor injection

In a study conducted by Machado et al. (2023a) among 79 patients who underwent SLNB, the recognition rate of trans-tumor percutaneous injection of UCAs for SLNs could reach as high as 88.50%, which is higher than that of the radiotracer (88.10%). More importantly, in 34 cases of pathologically diagnosed positive SLNs, the detection rate of CEUS was found to be 100.00% (34/34). NAC kills cancerous tissues while also being destructive to normal tissues, including lymphatic vessels, interfering with the detection of lymph nodes. According to further analysis by Machado et al. (2023b), trans-tumor peritumor injection of UCAs was a better method in NAC patients, with an accuracy of 53.00% for SLNs and keeping a 100.00% detection rate for positive lymph nodes (15/15). This demonstrates that the method of peri-tumor injection of UCAs has high accuracy and stability in the identification of SLNs, and it is especially suitable for surgical patients after NAC.

5.4 Other injections

SLNs have been traced using intravenous UCAs in a number of investigations. Malignant tumors have a good blood supply, which can present high signals more quickly under CEUS. Besides, the enhancement pattern can be used to determine the quality of the tumor (Lee SC et al., 2019). However, the UCA administered using the aforementioned method mostly remains in blood vessels and has a negligible content in the lymphatic vessels, making it tough to detect SLNs. Moreover, the lymph nodes displayed by this method are most likely not the first-station lymph nodes but instead could be the second- or third-station lymph nodes (Du et al., 2008; Caproni et al., 2010). In fact, few studies have been written concerning intravenous UCAs, prompting further research.

5.5 Comparison of various injection methods

Relative to capillaries, lymphatic capillaries have a higher permeability for UCAs. The superficial layer of the breast skin has a higher density of lymphatic vessels, and the lymphatic capillaries under the areola are the beginning of breast lymphatic drainage, making it easier for UCAs to access these capillaries. After the subcutaneous injection of UCAs into the areola, the lymphatic vessels and lymph nodes are plainly visible, unaffected by the blood vessels. Subcutaneous or intradermal injection of UCAs around the areola is easy to accomplish, and thus this is suitable for tumors in various locations and has a high value in clinical application (Omoto et al., 2009). Additionally, it is thought that the detection rates of SLNs by the breast areola injection UCAs and the blue dye method are similar and occasionally greater than that of the MB. After performing 700 SLNBs, Bass et al. (1999) concluded a constant location of the SLN: in the outer upper quadrant of the breast over the lateral border of the pectoralis major muscle near the axilla. Thus, the lymphatic drainage of the breast can be regarded as a whole unit, as the majority of the lymph (more than 75%) drains to the same SLN. As a result, whether an injection is made intradermally or subcutaneously around a tumor or areola, a similar result is achieved. The peritumor injection enables the UCA to enter lymphatic vessels that are connected to the tumor, exposing matching SLNs that are more representative of the status of the axillary lymph nodes. Nevertheless, this conjecture still needs to be verified by further experiments. The intravenous injection method will reduce the detection rate, since UCAs are disseminated within the systemic circulatory system and the dose in the tumor area is low, resulting in SLNs not easily detected by the ultrasonography detector.

In summary, the preferred method of CEUS is subcutaneous or intradermal injection in the areola, followed by the peri-tumor injection method, while the trans-elbow vein injection method is considered inappropriate. Fig. 4 illustrates a subcutaneous injection of UCAs through the areola to tracer SLNs in a clinical setting and localization with a metal wire.

6 Exploration of the optimal injection dose for CEUS

6.1 Injection dose of CEUS

The dose of injection could affect the detection rate of SLNs, and adopting the optimal dose may not only reduce the occurrence of adverse reactions but also account for the high detection rate. In a recent multicenter clinical study on the localization of SLNs by CEUS in China, it was found that the intracutaneous injection of 0.6 mL of UCAs at 3, 6, 9, and 12 o'clock positions via the areola was effective in improving the sensitivity and specificity of the diagnosis of SLNs (Li et al., 2022). With the same method and dose, Hu et al. (2020) found that CEUS had a 95.30% detection rate for SLNs. Furthermore, Liu et al. (2019) performed CEUS after subcutaneous injection of UCA (SonoVue, total dose 2.0 mL) around the areola of the affected breast, and they also successfully found SLNs with an accuracy rate of 94.67% (71/75) compared to MB. Luo et al. (2021) performed CEUS in 390 breast cancer patients scheduled for SLNB by injecting 1 mL of UCAs intracutaneously or subcutaneously at the 3, 6, 9, and 12 o'clock positions in the affected areola. Their analysis revealed that the recognition rate of CEUS for SLNs was 95.64% (373/390), which was higher than that of MB (92.05%).

6.2 Toxic effects of CEUS

UCAs are not only affordable and simple to use, but they also have fewer negative effects. After injecting

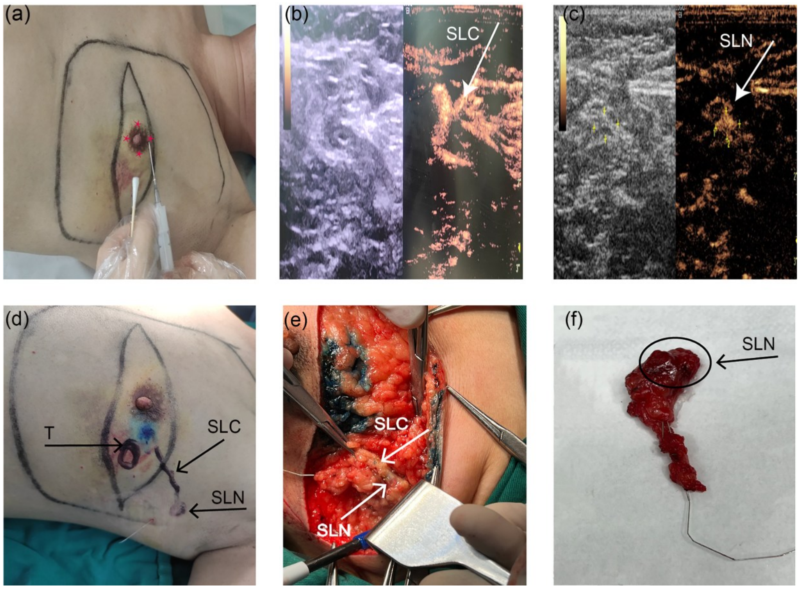

Fig. 4 Trans-areolar subcutaneous injection of ultrasound contrast agents (UCAs) tracing sentinel lymph nodes (SLNs). (a) Subcutaneous injection of SonoVue at the 3, 6, 9, and 12 o'clock positions in the affected areola. (b) Enhanced image of sentinel lymphatic channel (SLC). (c) Enhanced images of SLN. (d) Skin surface markers of tumor (T), SLC, and SLN. (e) Intraoperative methylene blue (MB) development of SLC and SLN. (f) The SLN of contrast-enhanced ultrasound (CEUS) wire positioning is consistent with the MB positioning.

different doses of UCAs into 12 female volunteers, Machado et al. (2018) found that the injection of 1 and 2 mL UCA yielded the same imaging rate for SLN. Some patients experienced only minor discomfort, including pain and nausea, and no serious adverse reactions were observed. Likewise, Shimazu et al. (2017) conducted a multicenter clinical study of CEUS searching for breast cancer SLNs. No related adverse events, such as skin reaction and allergic reaction, were observed after 3–6 months of follow-up. However, it is important to note that CEUS should be avoided in patients with the following conditions: (1) female patients who are pregnant or still breastfeeding; (2) patients with recent acute coronary syndrome or abnormal pulmonary function; (3) patients with severe pulmonary hypertension, i.e., pulmonary artery pressure of 90 mmHg (1 mmHg=0.133 kPa) or more (Schwarze et al., 2020; Golemati and Cokkinos, 2022; Safai Zadeh et al., 2022).

In conclusion, for the visualization of SLNs in breast cancer, 2 mL is the optimal dose of UCA. Whether injected at the same site or separately, it ensures a high visualization efficiency while minimizing the incidence of adverse effects in patients. This injection dose is consistent with the dosage used in our hospital.

7 Prospects

There are numerous tracers available for axillary SLN search, each one having its associated drawbacks. For instance, MB is among the most commonly utilized tracers, whose effectiveness is directly correlated with operators' surgical expertise and proficiency. If the surgeon does not perform a thorough search, SLNs are easily missed. Moreover, the lack of ALND will cause delayed treatment if the SLN has metastasized.

Despite the fact that CEUS is more operable than other dyes, it requires doctors to perform related operations after anesthesia, which increases the anesthesia time. Therefore, using CEUS and metal wire positioning before operation can shorten anesthesia time, lower the likelihood of missed detection, lessen damage surrounding tissue caused by lymph node searching, and improve patient prognosis. Besides, it simplifies operation so that even novices can perform it. At present, the research on CEUS is mainly retrospective analysis, with few prospective studies performed to date. In our single-center prospective clinical research, we attempted to perform SLNB under CEUS and wire localization before surgery. Many of the SLNs found by CEUS were discovered to be basically the same as MB development; this procedure can shorten the length of the axillary incision during breast-conserving surgery for obese women. This was established by a reduction in the false negative rate of SLNB and accurate localization of SLNs. In addition, based on the excellent targeting characteristics of UCAs, we speculate that relevant therapeutic agents can be added to the UCAs to target tumors (Wang and Zheng, 2019). In addition to the promising future of CEUS in SLN tracing, several researchers have revealed that both benign and malignant lymph nodes can be identified with this technique in advance based on the lymph node enhancement mode. It is anticipated that CEUS will replace the currently used tracer and become a key tool for evaluating the status of preoperative lymph node metastasis.

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Author contributions

Qiuhui YANG: writing – original draft, conceptualization, and formal analysis. Yeqin FU: investigation and writing – review & editing. Jiaxuan WANG: software and visualization. Hongjian YANG: conceptualization, supervision, and writing – review & editing. Xiping ZHANG: conceptualization, funding acquisition, supervision, and writing – review & editing. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Qiuhui YANG, Yeqin FU, Jiaxuan WANG, Hongjian YANG, and Xiping ZHANG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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