Identification of Sentinel Lymph Nodes Using Contrast-Enhanced Ultrasound in Breast Cancer

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Abstract Sentinel lymph node (SLN) biopsy has become the standard of care in axillary staging of breast cancer patients who are clinically node negative as it reduces the morbidity of axillary nodal dissection. SLN biopsy using blue dye and radioisotopes have high identification rates but its limitations include anaphylaxis, disposal of radioactive waste, and potential second surgery in up to 35% of patients who show nodal metastases on SLN biopsy. Contrast-enhanced ultrasound (CEUS) has the potential for SLNs to be identified without the aforementioned risks. CEUS involves the administration of intravenous contrast agents containing microbubbles of perfluorocarbon or nitrogen gas. The bubbles greatly affect ultrasound backscatter and increase vascular contrast in a similar manner to intravenous contrast agents used in CT and MRI. It is safe and easily performed with no requirement for ionizing radiation and no risk of nephrotoxicity. Microbubbles are taken up by lymph nodes when injected directly into tissues, including sub-areolar injection in the breast cancer patient. This method may prove valuable in patients with ductal carcinoma in situ, where operative SLN biopsy remains controversial, and in women undergoing prophylactic mastectomies for high risk. This technique may also have a role after neoadjuvant chemotherapy where frequently there is fibrosis in the treated SLNs.

Keywords Sentinel node biopsy · Contrast-enhanced ultrasound · Breast cancer

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Introduction

The detection or exclusion of lymph node micrometastases is critical in staging breast cancer because it directly affects patient prognosis and surgical management [1, 2].

Current guidelines recommend completion axillary lymph node (ALN) dissection when the sentinel lymph node (SLN) biopsy reveals macro- or micrometastases (>0.2 mm) [3]. Approximately 50% of patients with positive SLN biopsy findings harbor additional non-sentinel ALN metastases. This possibility represents an important drawback of the SLN biopsy procedure because these patients normally require a second operation. More accurate preoperative staging would reduce this need.

SLN biopsy has become the standard of care in axillary staging of breast cancer patients who are clinically node negative because it reduces the postoperative complications and morbidity of axillary nodal dissection [4, 5].

The SLN is defined as the first node in the regional nodal basin that receives lymphatic drainage from the primary tumor. The breast has a simplified lymphatic drainage system, with axillary SLNs receiving afferent lymphatics from the breast parenchyma and the sub-areolar plexus.

Currently, intraoperative SLN biopsies using blue dye and radioisotopes have high identification rates [6, 7]. The limitations include anaphylaxis [8], disposal of radioactive waste, and potential second surgery in up to 35% of patients who show nodal metastases on SLN biopsy [9].

Pilot studies on non-invasive in vivo SLN mapping are important to allow pre-treatment staging of the axilla in patients who are candidates for neoadjuvant chemotherapy and endocrine therapy.

Metastatic lymph nodes exhibit peripheral and mixed vascularity because of the tumor angiogenesis.

Color Doppler ultrasound scan (US) can only provide information regarding the macrovessel flow and morphology; therefore, it is difficult to accurately diagnose lymph node metastasis using this method.

Sonazoid, a new generation contrast agent for US, allows for visualization of lymph node microvessels.

Compared with previously used imaging modalities, contrast-enhanced ultrasound (CEUS) with Sonazoid would be expected to result in more accurate evaluation of lymph node metastasis.

This method may prove valuable in patients with ductal carcinoma in situ, where operative SLN biopsy remains controversial, and in women undergoing prophylactic mastectomies for high risk.

This technique may also have a role in patients with downstaging of tumors after neoadjuvant chemotherapy and those who frequently show fibrosis in treated SLNs.

Finally, contrast-enhanced ultrasound may allow SLNs to be identified without radiation exposure to the patient, surgeon, and support personnel.

Gray-scale and power Doppler axillary ultrasound are established diagnostic tools for evaluating the axilla by measuring anatomic features and observing blood flow non-invasively but are unable to show SLNs.

Current limitations of in vivo gray-scale ultrasound of the axilla include spatial resolution and ultrasonic resolution in vivo, which pose challenges for imaging deep (20–30 mm) SLNs.

The technology of microbubbles as an ultrasound contrast agent is based on the concept of encapsulated gas bubbles that are smaller than red blood cells (RBCs), circulate freely in the body, and are thus labelled blood pool agents.

First-generation high-mechanical-index contrast agents that were based on air-filled microbubbles were limited by the short transit time of the signal.

Second-generation contrast agents that are detected by gray-scale harmonic imaging techniques allow real-time high-resolution imaging of both the arterial and parenchymal phases and allow visualization of diffuse and partial alterations of nodal perfusion in lymph nodes that are sub-centimeter in size.

This technique provides intriguing information on nodal perfusion. There are currently no methods of targeting SLNs using IV administration of microbubbles.

Microbubbles are taken up by lymph nodes when injected directly into tissues, including sub-areolar injection in the breast cancer patient.

The study by Sever et al. [10] shows that SLN detection with contrast-enhanced ultrasound is feasible.

Significant advantages of ultrasound include wide availability, low cost, ease of utilization, rapid study, and real-time imaging capabilities.

The true clinical challenge is to evaluate whether the use of ultrasound contrast materials has a role in delineating intra-

nodal morphology (perfusion defects) that may help differentiate benign from malignant disease in vivo, as was reported by Goldberg et al. [3] in animal models (95% sensitivity, 19/20 SLNs with metastases; 63% specificity, 5/8 SLNs negative for metastases).

Abnormal perfusion defects identified in vivo can be targeted for needle biopsy to stage the axilla more accurately preoperatively.

Patients with biopsy-proven metastases will therefore not require surgical SLN biopsy and can proceed directly to axillary nodal dissection.

Material and Methods

This prospective study was approved by the Ethics Committee. All patients gave written informed consent.

Between November 2012 and December 2015, 50 consecutive consenting patients with clinically node negative breast cancer who were scheduled for primary surgical treatment were recruited into the study. Patient and tumor characteristics are shown in Table 1.

Exclusion criteria were histologically or cytologically proven ALN involvement diagnosed clinically or using standard sonographic imaging and the presence of severe medical comorbidities.

Table 1 Patient and tumor characteristics

Baseline characteristics	Number of patients (N = 50)
Patient characteristics	
Menopause status	
Premenopausal	29 (58%)
Postmenopause	21 (42%)
Tumor characteristics	
Orientation	
Upper outer quadrant	29 (58%)
Upper inner quadrant	12 (24%)
Lower outer quadrant	3 (6%)
Lower inner quadrant	3 (6%)
Central quadrant	3 (6%)
Tumor size	
T1	12 (24%)
T2	32 (64%)
T3	6 (12%)
Histology	
Invasive ductal	46 (92%)
Invasive lobular	4 (8%)
Stage	
0	0 (0%)
I	16 (32%)
II	31 (62%)
III	3 (6%)
Molecular sub-type	
Hormone positive	28 (56%)
Hormone negative	13 (26%)
Her-2 positive	9 (18%)
Her-2 negative	41 (82%)
Triple negative	9 (18%)

All sonographic examinations were performed by a single experienced breast radiologist using an Acuson Sequoia 512 scanner (Siemens Healthcare). This scanner provides conventional gray-scale imaging, pulse-inversion harmonic gray-scale imaging, and contrast-specific sonographic imaging.

All patients with breast carcinoma underwent axillary sonography as part of their initial assessment. Patients with abnormal or suspicious nodes underwent fine-needle aspiration cytology (FNAC) or core biopsy (CB).

All patients with normal findings on axillary sonography or negative axillary biopsy results were assessed clinically, and patients suitable for routine SLN biopsy were invited to be included in this study.

For consenting patients, in order not to delay the surgery, axillary assessment with microbubbles was performed 1 day prior to the surgery. The median tumor size was 26 mm (range, 15–120 mm).

There were 44 cases of invasive ductal cancer and 6 cases of invasive lobular cancer. Lymphovascular invasion was seen in 17 cases (34%). The lesions were located in the breast as follows: 29 in the upper outer quadrant, 12 in the upper inner quadrant, 3 in the lower outer quadrant, 3 in the lower inner quadrant, and 3 in the central quadrant.

The Microbubble Procedure

Before microbubble injection, 1 mL of 1% lidocaine was injected into the subcutaneous layer of the areola.

The microbubble contrast agent was reconstituted using 2 mL of sterile saline, and the ampoule was shaken vigorously to ensure a homogeneous suspension of bubbles.

The ultrasound contrast agent is composed of phospholipid-stabilized microbubbles containing sulfur hexafluoride gas (SonoVue) with a mean diameter of 2.5 μm .

Between 0.2 and 0.5 mL of the microbubble suspension was injected into and just under the skin of the areola in the upper outer quadrant using a 1-mL insulin syringe and a 26-G needle.

Up to three consecutive injections were given intradermally similar to the technique used for radioisotope and blue dye injections in patients undergoing conventional SLN biopsy.

A conventional gray-scale ultrasound examination of the axilla was performed before injection of microbubbles, and the positions of the imaged lymph nodes were noted.

After injection of the microbubbles, lymphatic channels were visualized immediately on contrast pulse sequencing and were tracked into the axilla.

Areas of contrast agent accumulation were then imaged with gray scale or were simultaneously viewed on live dual images to confirm the presence of an architecturally defined lymph node. When no obvious lymphatic filling was seen, the areola area was massaged for 10–30 s and imaging was repeated. If no lymph nodes could be identified after three consecutive injections, the study was abandoned and recorded as a failure.

In patients whose nodes were successfully identified, the transit time from injection to arrival in the axillary node was between 15 and 45 s. Contrast agent remained in the presumed SLN for up to 3 min. Repeating the areola massage often produced a refill of the SLN without the need for a second injection.

When an enhancing node was identified in the axilla, a guide wire was placed into the node. In most cases there was only one SLN; if two SLNs enhanced simultaneously, both

Fig. 1 **a** Contrast-enhanced ultrasound image obtained using contrast-specific mode reveals nodal enhancement (arrows). **b** Conventional gray-scale image shows the same node (arrows) as **a**. **c** After the sentinel lymph node was identified with microbubbles, a guide wire was placed into this node (short arrows) using 20-G needle (long arrows)

were marked. A 7-cm-long double hook guide wire (20 G, BARD) was used to localize the sentinel lymph nodes. Once identified, contrast-enhanced ultrasound-identified SLNs were localized with double-hook guide wire (Fig. 1).

After insertion, the tails of the guide wire were covered with a sterile dressing. Considering the unavoidable frequent movements of an upper limb, and the discomfort caused by a foreign matter in the axilla, it was more appropriate to perform this procedure the evening before the surgery.

There was no displacement of wires, hematoma, or infections.

Sentinel Lymph Node Biopsy

At surgery a standard mastectomy incision was made, and the marked nodes biopsied and sent for frozen section and imprint smear examination.

Immediate ALND was carried out if the SLN (i) was reported positive on intraoperative assessment or (2) could not be identified. Delayed ALND was done if an initially reported negative SLN turned out to be positive on paraffin section examination.

The pathologic assessment of the SLNs was performed according to the *Sentinel lymph node biopsy handbook: new start guidelines* [11].

The pathologist also noted whether there was evidence that the SLN had previously been marked with a guide wire by noting the presence of a linear tract of granulation tissue with associated hemorrhage, fat necrosis, or hemosiderin-laden macrophages.

Results

A total of 50 patients who had a microbubble injection and underwent SLN biopsy were included in the assessment. There were 50 women and no male with a median age of 42 years (range, 36–70 years). Of these, five patients had screening mammography-detected cancers, and the remaining 45 cases were symptomatic referrals to the breast clinic.

There were no axillary hematomas found after placement of guide wires.

The microbubble procedure was defined as successful when the following three parameters were met:

- (i) Lymphatic enhancement identified at the site of microbubble injection and followed into the axilla where enhancing lymph nodes were seen
- (ii) A successful biopsy of these nodes
- (iii) Pathologic confirmation of the guide wire tract within the excised SLNs

When biopsy of the presumed SLN yielded positive findings, patients proceeded to ALN dissection and these cases were also regarded as successful.

The procedure was unsuccessful in six patients. In two patients, microbubble injection failed to show any enhancing lymph nodes; therefore, no biopsy was performed. These patients underwent standard ALND. In 1 case, the lymphatics were seen but could not be followed into the axilla. In the one remaining case, no lymphatics were identified.

Four biopsies were reported as suspicious but not confirmed on frozen section examination. Those cases were also recorded as technical failures. Three of these 4 failed cases (75%) were positive at subsequent axillary dissection. In 1 case, all nodes were negative after ALND and histopathologic examination.

The overall results of the procedure are shown in Table 2.

The microbubble procedure was deemed successful in 48 of the 50 cases (96%) when the SLNs were correctly identified and biopsied. In 33 of the 44 (75%), frozen section examination of the SLN biopsy sample from the microbubble procedure proved a metastatic deposit in the SLNs and these patients underwent ALN dissection section (true-positive biopsy). In 11 cases there was no evidence of malignancy within the SLN biopsy specimen and in these patients ALND was not done at the time of primary surgery (Fig. 2).

In 07 cases the biopsied node was the only positive node in the axillary specimen and in 03 of those seven cases the only evidence of axillary disease was a micrometastasis (0.2–2.0 mm) in the biopsied node. The other 26 patients had more than one involved node in their axillary dissection specimen (Fig. 3).

Table 2 Overall results of the procedure

Variable	Value
Total number of cases	50
Technical failures	6
No lymph node identified	2
No confirmation (but suspicion) in excised SLNs (also included in technical failure)	4
Technical success	44
True-positive results (33/44)	33
False-positive results	0
True negative results (09/11)	9
False-negative results (02/11)	2
Sensitivity (%) (33/35)	65%
Specificity (%) (09/09)	100%
Positive predictive value (%) (33/33)	100%
Negative predictive value (%) (09/11)	81.81%
Accuracy (42/44)	95.45%

Fig. 2 Photomicrograph (H & E) of histopathologic slide from sentinel lymph node specimen of a 46-year-old lady with grade II invasive ductal carcinoma. Micrometastasis (1.4 mm) was identified within the node (arrowheads). Hemorrhage from guide wire tract was noted a few millimeters away from metastatic deposit (arrows)

Four biopsies were reported as suspicious but not confirmed on frozen section examination. Those cases were also recorded as technical failures.

In 11 cases, there was no evidence of malignancy within the SLN biopsy specimen and in these patients ALND was not done at the time of primary surgery. In 2 of the 11 cases (18%), there was malignancy in the paraffin section examination (false-negative biopsy) and these patients underwent completion ALND at a second surgery. In one of the two patients who underwent completion ALN dissection, there were no other involved nodes identified in the axillary specimen. In the remaining one patient, however, the completion ALN dissection specimen contained macrometastatic deposits in additional 3 of 11 non-SLNs.

Fig. 3 SLN biopsy in a 37-year-old lady with stage I disease undergoing BCS. The guide wire containing SLNs excised (single arrow), labelled, and sent for histopathology. Adjoining node that was suspicious-looking adjoining node were also marked

In most of the cases, the lymphatics were readily followed into the axilla and microbubbles highlighted, by their enhanced reflection, a single axillary node. However, in some cases, a second or third node was also noted enhancing subsequently via the efferent lymphatics connecting the first node to the others.

In six of the 50 patients (12%), two nodes were identified by simultaneous enhancement and both nodes were biopsied. Three patients in this group were proven to have axillary metastasis and all were treated with immediate ALN dissection.

In 16 of the 50 patients (32%), the enhancing lymph node or nodes were also seen on gray-scale imaging before the microbubble injection. However, in 32 cases (64%), the presumed SLN could be identified as a lymph node by sonography only after the microbubble enhancement.

It was otherwise not visible on gray-scale imaging either because of its deep location or because it had a very thin cortex and a prominent fatty hilum and therefore was obscured by the surrounding axillary fat.

Discussion

SLN biopsy has become the routine procedure in patients with early breast cancer, and axillary lymph node dissection (ALND) is the standard treatment for SLN-involved case.

According to the ACOSOG Z0011 and IBCSG 23-01 data, patients with limited SLN metastatic breast cancer treated with breast conservation and systemic therapy may omit ALND [12, 13], but these conclusions still need to be validated by more well-designed trials. So the primary value of SLN biopsy remains indicating axillary status with minimal trauma.

The combination of radioisotopes and blue dye is a popular method for SLN tracing. However, their limitations are not negligible.

The diameter of radioactive colloid is usually between 50 and 100 nm, while the endothelial gap of subcutaneous lymph vessel is between 120 and 500 nm; so the radioactive colloid cannot pass through the lymph vessel freely but can be absorbed by endocytosis. Therefore, many hours are needed to detect SLNs after radioactive colloid is injected.

Moreover, radioactive materials handling is another challenge for hospital management. A blue dye may lead to staining of adjacent adipose tissue. Anaphylaxis following blue dye injection is also an infrequent but serious risk for patients.

In this study, we chose sulfur hexafluoride microbubbles as a contrast agent, which is much smaller than red blood cell; so the agent can readily cross into the SLNs and be easily eliminated. The detecting instrument is a normal ultrasound apparatus with contrast pulse sequences, and it is readily available. Moreover, there are no iodine and proteins in sulfur hexafluoride microbubbles, which safeguards patients from allergy.

The potential application of CEUS and microbubbles in identifying SLNs in a swine melanoma model was first reported in 2004 [3]. Then, a few studies of SLN identification by CEUS and microbubbles in human beings were published.

A UK study with 54 patients found 89% identification rate by CEUS and intradermal microbubbles [14]. This result was repeated in a further 80-patient UK study [10].

SLN biopsy using blue dye and radioisotope often results in the removal of multiple SLNs. In this study, the identification rate of CEUS was 95.45%.

The characteristic advantage of CEUS and microbubbles is the real-time imaging for SLNs. A dynamic process of SLN enhancement from the afferent lymphatic vessel to efferent vessel is visible, and the very first developed lymph nodes are easily identified and located with a guide wire. Therefore, the number of SLNs detected by CEUS is less than that reportedly detected by blue dye.

In other words, SLN biopsy with CEUS can adequately stage the axilla with fewer traumas. The false-negative rate of SLN biopsy is the most important concern of the surgeons. It was reported that SLN biopsy had an inherent false-negative rate in the range of 4–10% [15, 16]. A similar drawback was recognized by Britton et al. [17] when half of the false-negative cases revealed a biopsy tract within the SLN. It might be possible to overcome this sampling error by removing the entire contrast-identified lymph node for detailed histologic analysis.

Breast lesions are increasingly being removed under sonography guidance using vacuum-assisted and other devices. It is possible that similar techniques could be used to remove the entire SLN. This would have the potential to obviate conventional SLN biopsy and replace it with a percutaneous outpatient procedure.

In our study, all the axillary positive cases were diagnosed correctly by CEUS. In two patients, the paraffin examination of the sentinel node subsequently came positive for malignancy, and therefore, these two patients required completion surgery and had delay in their treatment. There was no unnecessary ALND which is noteworthy. In two patients, microbubble injection failed to show any enhancing lymph nodes, and therefore, no biopsy was performed.

It is well established that lymph nodes totally replaced by metastases can be associated with a false-negative SLN biopsy because these nodes may fail to take up blue dye or radioactive isotope when the lymphatics are obstructed [18].

In such cases microbubble enhancement may also fail. In patients who have no enhancing nodes, cautious gray-scale imaging—particularly at the site where abrupt lymphatic visualization stops—may help to identify abnormal lymph nodes. Such cases also highlight the importance of having an experienced surgeon who can recognize these involved nodes by the naked eye at operation.

Experience has shown one of the main limitations of gray-scale sonography is that the SLN can be obscured

within the axillary fat. In our study, 64% of the SLNs could be identified as a lymph node only after microbubble enhancement.

These results are also consistent with those of Britton et al. [17]; those investigators found that gray-scale sonography-guided biopsy missed the SLN in 36% of patients and concluded there was a need for more reliable methods of nodal identification.

The use of microbubbles and CEUS is of value in the diagnosis of involved SLN.

The normal direction of internal drainage of lymph nodes is from cortex to cortex, which appeared to be enhanced from peripheral region to central zone in CEUS. SLNs that contained US-detectable metastases demonstrated areas did not enhance as a result of tumor displacement or destruction of the normal tissue.

Goldberg had reported that the sensitivity and specificity of CEUS in predicting SLNs metastases in a swine model were 95 and 63%, respectively, with an overall accuracy of 86% [19]. In another 20-case study, inhomogeneous enhancement was also observed in 2 metastasized cases.

Peripherally increased echogenicity or longer enhancing period was observed because of the replacement of the inner portion of the LN by metastatic lesions [20]. However, there are few reports about the value of the CEUS in predicting lymph node metastases.

In our study, we obtained an accuracy rate of 95.45%, compared with the pathology result. This may be a valuable addition to the technique. The avoidance of a second operation to complete the ALN dissection should also have cost-saving implications.

A few limitations of this study merit comment. The data are from a single unit and a single radiologist. The procedure has a learning curve not only in identification but also in the biopsy technique. It is important to differentiate contrast pooling from actual nodal enhancement.

Conclusion

Microbubbles and CEUS is a feasible approach for SLN identification. The enhancing patterns on CEUS may be helpful to recognize the metastasizing SLNs. This novel method may be a promising technique for the clinical application.

There are concerns about the procedure being operator dependent, the cost of the microbubble contrast medium, and the discomfort to the patient from wire localization.

Larger patient data and multicenter cohort trials are required, and long-term follow-up data on the safety and therapeutic effect are still needed.

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