Improved False-Negative Rates Using a Novel Patient Selection Flowchart in Initially Biopsy-Proven Node-Positive Breast Cancer undergoing Blue-Dye Alone Guided Sentinel Lymph Node Biopsy After Neoadjuvant Chemotherapy

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Abstract

Purpose: Current trials support the application of sentinel lymph node biopsy (SLNB) in node-positive breast cancer treated with neoadjuvant chemotherapy (NAC) with a lower false negative rate (FNR) if dual-tracer (radioisotope and blue-dye) is used. However, radioisotopes are not available in many areas of the world. In this study, we evaluated the feasibility and accuracy of SLNB mapped with methylene-blue-dye alone.

Methods: This study enrolled 132 patients with biopsy-proven node-positive breast cancer with a clip placed in the positive node who then received NAC. After chemotherapy and before operation, all patients underwent axillary ultrasound (AUS) assessment and were classified as either negative (AUS-) or positive (AUS+) according to the axillary status. All patients underwent both SLNB and axillary lymph node dissection (ALND). SLNB was mapped with methylene-blue-dye alone. FNRs were evaluated on factors potentially affecting false-negative SLN finding.

Results: Using methylene-blue-dye alone, the FNR of SLNB was 9.9%. Post-NAC AUS assessment (p=0.009), number of SLNs retrieved (p=0.029), and the retrieved of the clipped node (p=0.086), showed association with FNRs in multivariate analysis. In AUS- group, FNR was as low as 2.5%. In AUS+ group, retrieving ≥4 SLNs including the clipped node improved FNR from 17.1% to 4.8%. A flowchart was designed with the combination of post-NAC AUS assessment, retrieved SLN number, and the retrieved of clipped node further improve overall FNR to 3.3%.

Conclusion: In biopsy-proven node-positive breast cancer treated with NAC, using a flowchart to optimize patient selection reduces the FNR of single-tracer (methylene-blue-dye) guided SLNB.

Introduction

Axillary lymph node status has been demonstrated to be an important prognostic factor for early breast cancer survival.[1–3] Sentinel lymph node biopsy (SLNB) is safe and accurate enough for axillary staging in initially clinically node-negative (cN-) breast cancer patients after neoadjuvant chemotherapy (NAC).[4–6] Approximately 36%-42% can achieve an axillary pathologic complete response (pCR) in initially node-positive breast cancer (cN+) after NAC, but SLNB still remains controversial for axillary staging in this population because of the low identification rates (IRs), high false negative rates (FNRs), and the lack of long-term regional recurrence data.[7–9] According to the results of several clinical trials that evaluated the accuracy of SLNB in initial cN + patients treated with NAC, the FNR would be improved in this setting with the use of dual-tracer (radioisotope and blue-dye), and obtaining ≥ 3 sentinel lymph nodes (SLNs) including the clip-marked positive lymph node confirmed pre-NAC.[4, 5, 10–15]

Lymphatic mapping using dual-tracer (blue-dye and radioisotope) for SLNB has been reported to decrease the FNR compared to single-tracer alone in biopsy-proven node-positive patients treated with NAC. However, this difference was not significant after multivariate analysis (p = 0.15) as reported in SENTINA clinical trial.[5] The use of radioisotopes causes some logistical challenges such as the

handling and disposal of isotopes, training of staff, and legal requirements, which have limited the worldwide adoption of SLNB for hospitals, with approximately 40% of an estimated 500,000 patients in developed countries having no access to the procedure.[16–18] Concern about the hazards of radiation exposure is also an obstacle for the use of the combined method. In China, dual-tracer (the combination of blue-dye and radiotracer or fluorescence) guided SLNB was reported to be performed in about 14.9% of breast cancer patients, while the majority of SLNBs were performed using a single mapping agent, including blue-dye, carbon nanoparticles, indocyanine green, or radiotracer.[19.20]

Despite theoretical concerns that using single mapping agents might impede the ability to detect SLN, some studies in which single mapping methods (radioisotope or blue-dye alone) were used to guided SLNB for cN + after NAC showed a similar IR (94.9–95.8% vs. 92.3–97.5%) but a higher FNR (22–36.4% vs. 7.7–16.0%) than that of dual lymphatic mapping.[5, 10, 21–25] However, few have focused on how to improve the FNR of single-tracer guided SLNB by combining with the FNR-improving methods proposed in previous studies, such as optimizing patient selection, marking the biopsy-proven positive node before NAC for excision and evaluation of the known metastatic node after NAC, and increasing the SLN number.

The goal of this study was to evaluate the feasibility and accuracy of SLNB using blue-dye alone in patients with biopsy-proven positive nodes treated with NAC. Additionally, we sought to determine the effect of axillary ultrasound after NAC, SLN number harvested during surgery, and the clipped node retrieved as SLN on the FNR of SLNB using blue-dye only.

Materials And Methods

Our prospective study cohort enrolled breast cancer patients who were biopsy-proven by pathology node positive and with a clip placed in the involved or metastatic node who were subsequently scheduled to undergo NAC between January 2017 to December 2020 at the Department of Breast Surgery, Fujian Medical University Union Hospital. The study was approved by Ethics Committee, and all the participating patients signed an informed consent.

Patients

All histologically proven primary invasive breast cancer (cT1-4, N1-3, M0) age 18 years or older with biopsy-confirmed nodal metastases were eligible for this study. The American Joint Committee on Cancer (AJCC) Staging Manual, 8th edition was used to determine the clinical and pathologic staging. Patients that did not complete the planned neoadjuvant regimens, those with pathological-confirmed distant metastases, pregnant or nursing women, and those with prior axillary surgery were excluded. We took an ultrasound-guided fine-needle aspiration, or a core needle biopsy of the most suspicious node determined by the radiologist after the examination of axillary ultrasound before NAC. If the node was confirmed to be metastatic, a titanium clip was placed in the biopsied node to mark it. The individualized neoadjuvant

regimes were based on anthracycline and/or taxane. HER2-targeted therapy such as Trastuzumab and/or Pertuzumab would be added for patients with human epidermal growth factor receptor 2 over-expression.

Nodal Assessment on Ultrasound after NAC

After completion of NAC and before an operation, all patients underwent an axillary ultrasound, conducted by two experienced radiologists, in our center to assess the residual disease in the nodes. Compared to normal lymph node (Fig. 1A), the node would be considered abnormal if; 1) the asymmetric cortical thickening ≥ 3 mm, 2) the fatty hilum was invisible, lost, or metamorphosed (Fig. 1B).[26, 27]

Surgical Procedure

Breast and axillary operations were planned to be performed at the same time within four weeks after NAC. Methylene blue-dye was injected alone peritumoral and/or subareolar 5–15 minutes before SLNB. All blue and suspicious palpable nodes were removed as SLNs, and then an axillary lymph node dissection (ALND) was performed in all patients, followed by the breast surgery. Radiographs of all removed SLNs specimen were performed for detection of clip-marked nodes. Radiographs of the whole breast and an axillary specimen were taken if the clipped node was not found. Each of the removed SLN was bisected and completely embedded into paraffin blocks each of which was then cut at three levels at a minimal 150μm intervals, and finally, stained with hematoxylin and eosin (H&E). Immunohistochemical keratin staining was only performed in negative SLNs cases with H&E staining, while positive nodes were detected in ALND specimens. Positive SLNs were defined as macrometastases, micrometastases, and isolated tumor cells (ITCs).

Statistical Analysis

The FNR was computed as the number of patients with negative SLNs who had residual disease in the contents of the ALND divided by the total number of patients with residual disease. Fisher exact tests were used to determine the likelihood of a false-negative SLN finding. We did multivariate logistic regression to find factors that affected the FNRs using SPSS 24 (SPSS Inc., Chicago, IL, USA). A p value lower than 0.1 was considered significant.

Results

A total of 142 breast cancer patients with T1-4, N1-3, M0 were enrolled into our study. Finally, 132 patients with at least one SLN were identified and included in the statistical analysis, given an IR of 93.0% for blue-dye alone guided SLNB. Clinicopathologic and treatment details of the 132 patients are listed in Table 1. The median age was 48 years (range 27–72 years). The majority of the 132 patients had ≥ cN2 disease (n = 73, 55.3%) before NAC, whereas 59 patients (44.7%) had cN1 disease. After NAC, 77 patients were classified as AUS- group by axillary lymph nodes morphologic appearance, while 55 patients were classified as AUS + group. The median number of SLNs was 4 (range 1–22), and 51.5% of patients had four or more SLNs identified. In the only patient with 22 sentinel lymph nodes removed, after ALND, there were 13 non-SLNs, and the 2 metastatic lymph nodes were located in SLNs, and one of them was clip-

marked SLN. The FNR of the clipped node alone is 10.0% (8/80). Among 117 patients with the clipped nodes as the SLNs, the FNR was 9.5% (7/74). Among 15 cases with the clipped node located in non-SLNs, 7 patients with the residual nodal disease and the FNR was 14.3% (1/7). And among 7 cases, 6 patients had positive SLNs, and 1 patient had negative SLN with negative clip-marked non-SLN. Thus, even if this case had the clipped node removed during SLNB, the false-negative rate would not be changed.

Table 1
Patient and tumor characteristics (n = 132)

Characteristic	No.(%)
Age, mean (range), y	47.6 (27–72)
Race	
Asian	132 (100)
Clinical T stage pre-NAC	
T1	11 (8.3)
T2	76 (57.6)
≥T3	45 (34.1)
Clinical N stage pre-NAC	
N1	59 (44.7)
≥N2	73 (55.3)
Receptor-based subtype	
HER-2+,HR-	29 (22.0)
HER-2+,HR+	28 (21.2)
TNBC	22 (16.7)
HER-2-,HR+	53 (40.2)
NAC regimen	
Anthracycline plus taxane	124 (93.9)
HER-2 + with single-targeted therapy	34 (25.8)
HER-2 + with dual-targeted therapy	23 (18.9)
Platinum for TNBC	15 (68.2)
AUS assessment post-NAC	
Normal (AUS-)	77 (58.3)
Abnormal (AUS+)	55 (41.7)
Type of breast surgery	

NAC, neoadjuvant chemotherapy; ER+, estrogen receptor positive; PR+, progesterone receptor positive; HER-2+, human epidermal growth factor receptor 2 positive; TNBC, triple negative breast cancer; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; AUS, axillary ultrasound; SLN, sentinel lymph node

Characteristic	No.(%)
Breast-conserving therapy	4 (3.0)
Mastectomy	128 (97.0)
No. of SLNs excised	
Median	4 (1–22)
1–2	38 (28.9)
3	26 (19.7)
≥4	68 (51.5)
Clipped-node & SLN	
Clipped-node as SLN	117 (88.7)
Clipped-node as non-SLN	15(11.4)

NAC, neoadjuvant chemotherapy; ER+, estrogen receptor positive; PR+, progesterone receptor positive; HER-2+, human epidermal growth factor receptor 2 positive; TNBC, triple negative breast cancer; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; AUS, axillary ultrasound; SLN, sentinel lymph node

Impact of patient and SLNB characteristics on FNRs

Residual nodal diseases had been examined in eight patients in ALND specimens who had negative SLNs, yielding an overall FNR rate of 9.9% (8/81; 95% CI, 5.1–18.3%). FNRs are analyzed by clinical and pathological characteristics in Table 2. Normal axillary lymph nodes assessed by post-NAC ultrasound, retrieving more SLNs, and retrieving the clipped SLN were more likely to have an improved FNR. The FNR was found to be as low as 2.5% (1/40; 95% CI, 0.4–12.9%) in the AUS- group compared than that of 17.1% (7/41; 95% CI, 8.5–31.3%) in the AUS + group.

Table 2
Impact of patient and sentinel lymph node (SLN) characteristics on false-negative rates (FNRs) in the entire cohort

Characteristics	FNR (%)	95% CI	P Value
Total	9.9 (8/81)	5.1–18.3	-
Clinical T stage pre-NAC			
T1-T2	5.7 (3/53)	1.9–15.4	0.12
≥ T3	17.9 (5/28)	7.9–35.6	
Clinical N stage pre-NAC			
N1	12.1 (4/33)	4.8–27.3	0.71
≥ N2	8.3 (4/48)	3.2–19.6	
AUS assessment post-NAC			
normal	2.5 (1/40)	0.4–12.9	0.05
abnormal	17.1 (7/41)	8.5–31.3	
No. of SLNs excised			
1–2 SLN	23.8 (5/21)	10.6–45.1	0.02
3 SLN	11.1 (2/18)	3.1–32.8	
≥4 SLN	2.4 (1/42)	0.4–12.3	
Position of clipped node			
Clipped node as non-SLN	14.3 (1/7)	2.6–51.3	0.53
Clipped node as SLN	9.5 (7/74)	4.7–18.2	
Clipped node & SLNs			
1–2 SLN,Clipped node as SLN	23.5 (4/17)	9.6–47.3	0.03
3 SLN,Clipped node as SLN	11.1 (2/18)	3.1–32.8	
≥4 SLN,Clipped node as SLN	2.6 (1/39)	0.04–13.2	
Receptor-based sub-type			
HER-2+,HR-	12.5 (1/8)	2.2–47.1	0.88
HER-2+,HR+	11.8 (2/17)	3.3–34.3	
TNBC	10.0 (1/10)	1.8–40.4	

SLN, sentinel lymph node; ER+, estrogen receptor positive; PR+, progesterone receptor positive; HER-2+, human epidermal growth factor receptor 2 positive; TNBC, triple negative breast cancer

Characteristics	FNR (%)	95% CI	P Value
HER-2-,HR+	8.7 (4/46)	3.4–20.3	

SLN, sentinel lymph node; ER+, estrogen receptor positive; PR+, progesterone receptor positive; HER-2+, human epidermal growth factor receptor 2 positive; TNBC, triple negative breast cancer

In multivariate analysis, clinical T status, clinical N status, or receptor-based sub-type showed no effect on the FNR. The AUS assessment after NAC (p = 0.009), the number of SLNs excised (p = 0.029), and the retrieved of the clipped node (p = 0.086) were associated with the FNR. Even in multivariate stepwise regression analysis, AUS assessment after NAC (p = 0.015) and the number of SLNs excised (p = 0.015) still affected the FNR (Fig. 2).

Impact of factors on FNRs for patients with either normal or abnormal post-NAC axillary ultrasound

The FNRs were analyzed according to the nodal status by AUS after NAC. (Table 3) In the AUS- group, the FNR was as low as 2.5% (1/40; 95% CI, 0.4–12.9%). Patients with 1–2 SLNs retrieved had the same FNR as those with ≥ 4 SLNs (0% vs. 0%). However, the only one false negative case in the AUS- group had three SLNs retrieved, resulting in a FNR of 11.1% (1/9; 95% CI, 2.0–43.5%) for those with three SLNs retrieved. The FNR in the AUS + group was estimated to be as high as 17.1% (95% CI, 8.5–31.3%). However, the FNRs were found to be fewer as the number of retrieved SLN increased. Retrieving ≥ 4 SLNs decreased the FNR to less than 5% (4.8%, 1/21; 95% CI, 0.85 to 22.7) in the AUS + group.

Table 3
Impact of factors on false-negative rates (FNRs) for patients with either normal or abnormal post-NAC axillary ultrasound

	FNR of Normal axillary nodes (AUS-)	FNR of Abnormal axillary nodes (AUS+)
Total	2.5 (1/40)	17.1(7/41)
Clinical T stage pre-NAC		
T1-T2	0 (0/29)	12.5 (3/24)
≥ T3	9.1 (1/11)	23.5 (4/17)
Clinical N stage pre-NAC		
N1	0 (0/17)	25.0 (4/16)
≥ N2	4.3 (1/23)	12.0 (3/25)
Receptor-based sub-type		
HER-2+,HR-	0 (0/5)	33.3(1/3)
HER-2+,HR+	0 (0/11)	33.3 (2/6)
TNBC	0 (0/1)	11.1 (1/9)
HER-2-,HR+	4.3 (1/23)	13.0 (3/23)
No. of SLNs excised		
1–2 SLNs	0 (0/11)	50.0 (5/10)
3 SLNs	11.1 (1/9)	11.1(1/9)
≥4 SLNs	0 (0/20)	4.5(1/22)
Clipped node & SLNs		
Clipped node as non-SLN	0 (0/4)	33.3 (1/3)
1–2 SLNs,clipped node as SLN	0 (0/8)	44.4 (4/9)
3 SLNs,clipped node as SLN	11.1 (1/9)	11.1(1/9)
≥ 4 SLNs,clipped node as SLN	0 (0/18)	4.8(1/21)

NAC, neoadjuvant chemotherapy; AUS, axillary ultrasound; ER+, estrogen receptor positive; PR+, progesterone receptor positive; HER-2+, human epidermal growth factor receptor 2 positive; TNBC, triple negative breast cancer; SLN, sentinel lymph node

A flowchart to improve FNR in initial biopsy-proven node-positive breast cancer using single-tracer alone (blue-dye alone)

In our study, using blue-dye alone, the overall FNR for SLNB was 9.9% in node positive patients treated with NAC. In multivariate analysis, post-NAC AUS assessment, the number of SLNs retrieved, and the retrieval of the clipped node showed significance with FNRs. Patients were then classified into AUS-/AUS + groups after NAC, and FNRs were analyzed between the two groups. The AUS- group had a low FNR of 2.5% compared than that of 17.1% in AUS + group. Interestingly, the FNR in the AUS + group decreased to 4.8% when ≥ 4 SLNs, including the clipped node, were retrieved. Using this method, the overall FNR improved from 9.9–3.3% (2/61; 95% CI, 0.9–11.2%).

Based on the integration of our results with international standard guidelines, a flowchart was designed with the combination of nodal assessment by post-NAC AUS, the retrieved SLN number during surgery, and the retrieval of the clipped node to improve FNR for SLNB using blue-dye alone in this setting (Fig. 3). Patients with biopsy proven node metastasis who planned to have NAC had the positive node marked with a clip to assist in finding it during SLNB. After NAC patients were divided into AUS-/AUS + groups according to axillary residual disease assessed by ultrasound. During SLNB, this evaluation is adequate to avoid ALND if ≥ 2 negative SLNs were retrieved in the AUS- group or ≥ 4 negative SLNs in AUS + group.

Discussion

Our study showed an acceptable FNR of 9.9% for single tracer (blue-dye) guided SLNB in patients with initial biopsy-proven node-positive disease treated with NAC. Compared to the NSABP-B32 trial, the residual axillary disease for node-positive patients in neoadjuvant settings was resistant to chemotherapy, which will increase the regional recurrence rate.[28] Therefore, to further decrease the FNR in this setting, we tentatively designed a flowchart by marking a metastatic lymph node with a metal clip before NAC, assessing the axillary lymph node status using AUS after NAC, and retrieving as many SLNs as possible, including the clipped node, during the surgery. For patients who were AUS- (cN-) after NAC, without retrieving the clipped SLN or restricting the SLN number, the FNR with blue-dye guided SLNB was 2.5%. Additionally, for patients who were AUS+ (cN+), the FNR decreased from 17.1% to 4.8% when removing ≥4 SLNs, including the clipped node. With this flowchart, the overall FNR decreased from 9.9% to 3.3%.

In previous studies, using AUS alone to predict the axillary status in initial node positive patients after NAC showed a high FNR of 12.6-61.3%.[29-31] Recent literature has focused on post-NAC AUS assessment as a complementary tool in patient selection for SLNB. A study associated with an SN-FNAC trial showed that patients with no residual nodal disease identified by node morphology in AUS after NAC had a lower FNR than those with residual disease (2.7% vs. 10.8%).[32] However, there was no consistent criteria mandated among radiologists to classify nodes as being positive or negative. In our study, AUS was also used to assess axillary load after NAC. Patients were classified as AUS-/AUS+ according to node morphology whether the asymmetric cortical thickening ≥3 mm or the metamorphosis in the fatty hilum.[27] The AUS- group had a lower FNR of 2.5% compared to 17.1% in the AUS+ group. However, 25.5% of the patients in our study and 19.6% in SN-FNAC trial with AUS+ who achieved ypN0 finally

underwent unnecessary ALND. Therefore, another effective strategy is needed to decrease the FNR in patient with AUS+.

According to well-designed prospective trials, marking the pathologic metastatic node before NAC and retrieving the marked node during SLNB has been considered an effective method to precisely evaluate the status of the marked node for residual disease after NAC and to improve the FNR.[10,12,14] However, the FNR-improving effect of this method was not obvious in our study. Most patients recruited in our study had cN2-3 disease (55.3%). Patients with cN1 disease accounted for 44.7% in our study compared to 94.6% in the Z1071 trial.[10] It can be suggested that since this method can only mark one metastasis lymph node, when it is applied to patients with a heavy disease burden in the axilla at diagnosis, its ability to reflect the overall axillary status would be limited. As a result, the FNR-improving effect would be masked. Besides, it is reported that in the real world, the FNR of marking and removing a previously positive axillary lymph node for breast cancer after NAC ranges from 0% to 28.6%.[22-41] So this clipped-marked method may not decrease FNR in all populations. However, we found differences in the SLN FNR between those cases when the clip was identified in the SLN versus those cases with the clip identified in the ALND specimen, and this difference may be clinically relevant. And among 117 patients with the clipped node retrieved in a SLN, up to 90.6% (106/117) had metastatic disease or a chemotherapy response in the clipped node, which allows evaluation for the response to neoadjuvant chemotherapy in that specific node. So, the importance and necessity of marking the metastatic node before NAC cannot be ignored. Marking the positive node at the time of diagnosis of breast cancer is still a potentially useful tool for those patients receiving NAC.

Previous studies showed that the use of a dual-tracer may improve the low IR and high FNR associated with fibrosis of the lymphatic channels and altering patterns of lymphatic drainage after NAC, with the IR ranging from 92.3% to 97.5% and FNR ranging from 7.7% to 16.0%.[2,10,23-25,42] However, due to the lack of radioisotope availability, the single blue-dye method has been in widespread use, especially in developing countries, because it is safe, cheap and does not need the nuclear medicine department and gamma probes.[25,43-46] Using a single-tracer, some studies showed a similar IR (94.9% to 95.8%) but a higher FNR (22% to 36.4%) than that of dual lymphatic mapping in this setting.[21,22] Although, the IR is a crucial determining factor reflecting the ability to identify the SLNs for SLNB, a recent meta-analysis showed that there is no significant difference in IR when the SLN was tagged using the different mapping methods ($p = 0.55$).[47] A prior study at the Memorial Sloan Kettering Cancer Center supported that it was mostly the nodal status pre-NAC, not the SLNB technique, which affect the retrieval number of SLNs.[48] Moreover, the result of the ACOSOG Z1071 trial, which supported increasing the number of SLNs to improve the FNR, incentivizes surgeons to remove as many SLNs as possible to reduce FNR in real clinical practice.[10,47,49,50] Although our study has achieved an acceptable FNR (9.9%) for blue-dye guided SLNB, it is a single-center study with a small number of cases and lack of external verification. Hence, we tentatively designed a flowchart to optimize patient selection and improve FNR in patients with this condition with the assist of post-NAC axillary ultrasound. According to our study results, for AUS- patients after NAC, we suggest that retrieving ≥ 2 SLNs including the marked node during SLNB is

adequate. For AUS+ patients, retrieving ≥4 SLNs, including the marked node, may effectively and accurately evaluate the nodal status.

This study has a few limitations. Firstly, in our study, we did not have comparison groups using radioactive isotope or combined method (radioactive isotope and blue-dye) for SLNB, so we were unable to firmly determine whether single-tracer only (radioactive isotope or blue-dye) is feasible and accurate enough compared to dual-tracer. Secondly, compared to several other large sample studies, our research sample size is medium, with 142 patients enrolled. However, our study is exploratory and prospective, trying to determine the reliability of SLNB using a single tracer for initial node-positive breast cancer treated with NAC, and we are hoping to expand this practice to other institutions to determine if these results are reproducible in a community setting.

Conclusion

In summary, our study found that in biopsy-proven node-positive breast cancer teeated with NAC, a low FNR (3.3%) for blue-dye alone guided SLNB can be achieved with strict use of a flowchart combined with marking the positive node with a metal clip before NAC, AUS assessment after NAC, and retrieving the appropriate number of SLNs, including the marked node, during the surgery. We recommend that this flowchart be further evaluated before being applied in future clinical practice for use after NAC.

Statements And Declarations

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Competing Interests

All authors declare that they have no conflict of interest.

Author Contributions

Fangmeng Fu and Chuan Wang: funding acquisition**. Fangmeng Fu, Chuan Wang and Lisa K Jacobs:** Study conception and design. **Fangmeng Fu, Chuan Wang:** Project administration. **Minyan Chen, Shengmei Li, Jingjing Guo and Xuan Huang:** Acquisition of imaging data**. Minyan Chen, Shengmei Li,**

Wenhui Guo, Lili Chen, Yuxiang Lin: Acquisition of clinical data. **Huang Meng, Fangmeng Fu, Minyan Chen, and Li Shengmei:** Analysis and interpretation of data**. Fangmeng Fu, Chen Minyan, Li Shengmei, and Lisa K Jacobs:** Drafting of manuscript. **Lisa K Jacobs, Chuan Wang, and Fangmeng Fu:**Critical revision.

Data Availability

The datasets analyzed during our study are available from the corresponding author on reasonable request.

Ethics approval

The study was approved by Ethics Committee, and all the participating patients signed an informed consent.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publish

The authors affirm that human research participants provided informed consent for publication of the images in Figures 1(A) and 1(B).

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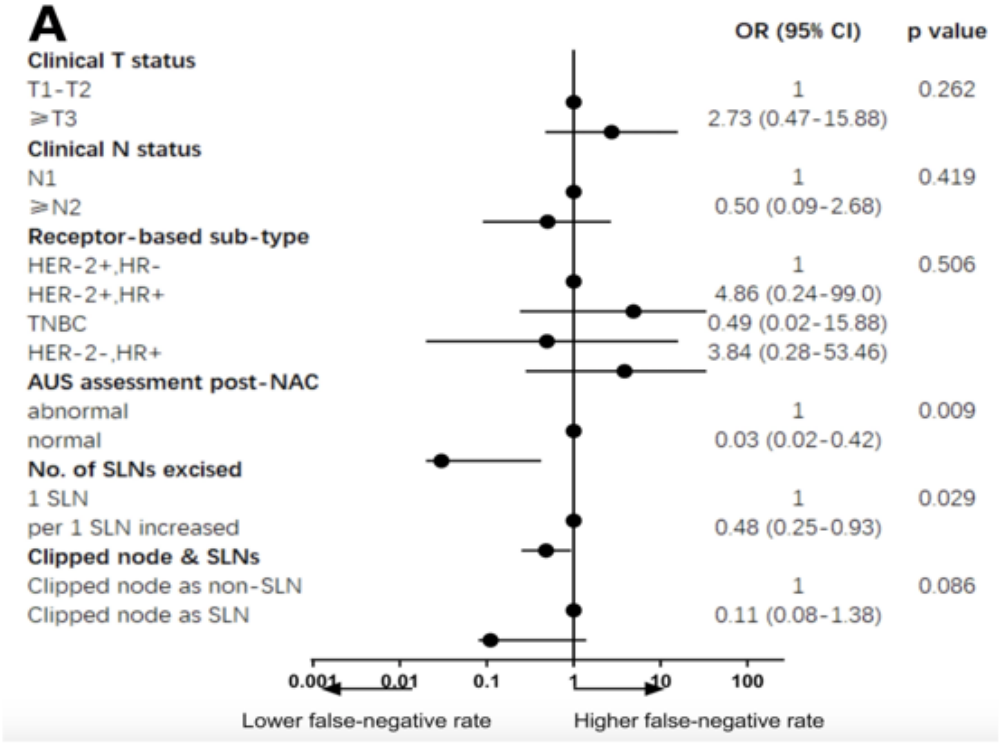
Figures

Figure 1

(A) Normal axillary node under US. The cortex was hyper-echoic and thin (3 mm thick) and the fatty hilum was visible; **(B)** Abnormal axillary node under US. Diffuse or focal asymmetric cortical thickening ≥ 3 mm and the metamorphose or invisible fatty hilum（the white arrow indicates the metamorphose fatty hilum,the blue arrow indicates the invisible fatty hilum）.



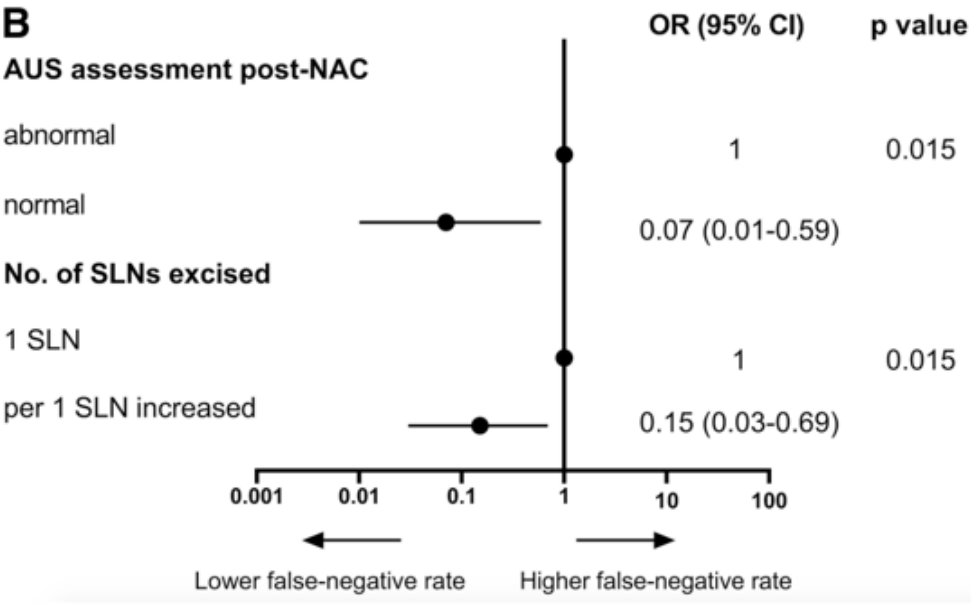


Figure 2

(A) Multivariate regression analysis for false-negative rates; **(B)** Multivariate step-wise regression analysis for false-negative rates.

Figure 3

Flowchart with the combination of nodal assessment by post neoadjuvant chemotherapy axillary ultrasound, the retrieved sentinel lymph node number during surgery and the position of clipped node.