Lymph node tattooing for targeted axillary dissection in postneoadjuvant chemotherapy breast cancer

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Introduction
Sentinel lymph node biopsy (SLNB) for patients with breast cancer with their axilla showing clinically node-positive disease (CN+ve) reduces morbidity and provides a higher quality of life by sparing axillary lymph node dissection for those patients whose axillary status changed from (CN+ve) to (CN0) after neoadjuvant chemotherapy. SLNB results are not encouraging in post-NACT setting; however, when it is combined with excision of previously marked positive LN, during targeted axillary dissection (TAD), identification rate (IR), and false-negative rate (FNR) are improved. The authors used suspended carbon particles (Blackeye ink) for preoperative LN marking, being a cheap alternative. Initial findings on TAD procedure following NACT on tattooed LNs and also the findings of SLNB by injecting 1% methylene blue (MB) were reported.

Patients and methods
A total of 40 patients with locally advanced breast cancer who were converted from cN+ to cN0 following NACT were divided into group A (20 patients), where TAD was performed and tattooed pathologically proven positive nodes besides SLNs were dissected, and group B (20 patients) for whom only SLNB using 1%MB was done. Backup axillary LN dissection was performed in all patients. The authors then compared IR and FNR in both groups.

Results
Blackeye ink was identified during surgery as a black stain on LNs. When the authors compared the results of TAD with SLNB, the authors found that diagnostic performance of TAD had the highest values, with FNR of 8.3% versus 15.3% in the SLNB group.

Conclusion
TAD using Blackeye ink and 1%MB has high IR and low FNR. It improves the accuracy of post-NACT axillary staging at very low cost.

Keywords:
breast cancer, methylene blue, sentinel lymph node biopsy, targeted axillary dissection

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Introduction

Neoadjuvant chemotherapy (NACT) is increasingly used for operable breast cancer. After NACT, node negative (cN0) conversion rate of 40–75% was reported for patients presenting with nodal metastases (cN+) [1]. Patients who achieved axillary pathological complete response had better 5-year overall (93 vs. 72%) and relapse-free (87 vs. 60%) survival rates than patients with residual nodal disease [2]. Axillary staging after NACT using sentinel lymph node biopsy (SLNB) could help the patient by avoiding axillary lymph node dissection (ALND). In patients with early-stage breast cancer with negative axillary nodes, SLNB by methylene blue (MB) is much cheaper than using radioactive isotope or the blue dye. The value of usage of SLNB in axillary staging after NACT for clinically node-positive disease (CN+) patients who converted to clinically node negative (CN0) has drawn a lot of controversy. Three prospective multi-institutional clinical trials (ACOSOGZ1071, SENTINA, and SN FNAC) assessed the accuracy of SLNB after NACT among these patients and reported identification rate (IR) between 87.6 and 92.9% [3–5]. In all of the studies, false-negative rates (FNRs) were greater than 10% and their end points were not met. A reduction of the FNR from 12.6 to 9.8% could be found in those patients with a normal axillary ultrasonography (US) after NACT and at least three removed SLNs. Axillary US suspects cN+ if lymph node (LN) cortical thickening (more than 2.5–3 mm) is present especially with compression or displacement of the hilum. On the basis of these findings, axillary US after NACT allows selection of

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patients with the greatest likelihood of being cN0, who will benefit from the omission of ALND and should be considered a standard procedure before SLNB [6]. According to the ACOSOGZ1071 study, in a group of patients with biopsy-proven positive LN that was marked with a clip prior to NACT, presence of the clipped LNs within the SLN removed dropped the FNR to 6.8% [5]. According to MARI trial, marking positive LN with I125 radioactive seed before NACT, then excision of the marked nodes after NACT completion without SLNB had only a 7% FNR [7]. A prospective trial that was done in a single institution excised clipped LN along with sentinel nodes and showed a FNR of 2%; accordingly, targeted axillary dissection (TAD) appears to be a reliable method for axillary staging after NACT for this group of patients [8]. The use of sterile black carbon suspension (spot IM) in tattooing biopsied Axillary Lymph Nodes (ALNs) was attempted for TAD procedure. The US Food and Drug Administration-approved spot im is a carbon-based marker that is popularly used for presurgical colonic tattooing, with the advantage of remaining identified for several months. The Blackeye ink is also similar to spot im [9]. In a previous study, Spotink was injected in the metastatic axillary LN before starting NACT; black tattoo ink was visualized intraoperatively in all cases, except one case with microscopic black pigment only. It was concluded that tattooing is a feasible and low-cost method for marking biopsied nodes. The black pigment could be discerned by surgeons from blue dye after finishing NACT. In addition, tattooed LNs correlate well with sentinel nodes, which add to the accuracy of surgical axillary staging [10]. We address the results on TAD procedure with MB tattooed LN after NACT-SLNB. The primary goal of this study was to determine the sentinel node concordance with marked nodes and the intraoperative IR of tattooed nodes. A secondary goal was to evaluate the procedure's FNR and see whether TAD enhances it as compared with SLNB alone.

Patients and methods

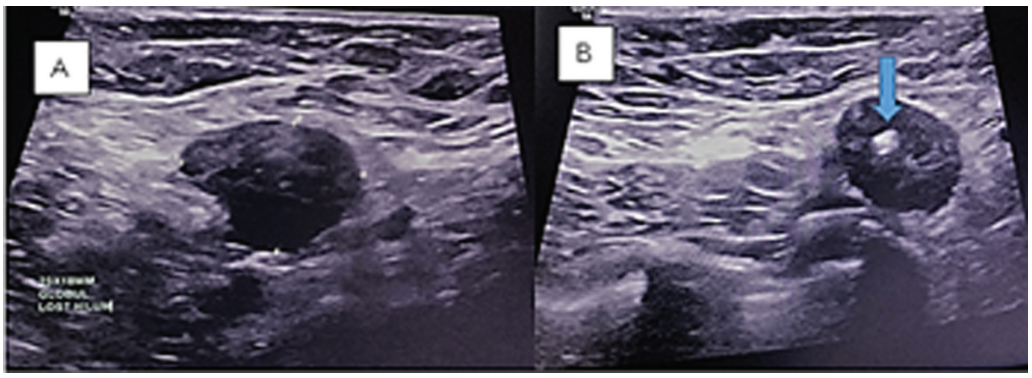
Between July 2017 and January 2020, 56 females with noninflammatory node-positive locally advanced breast cancer (T2-4, N1-3, M0) were evaluated after having an informed consent about the study and their treatment plan. The study protocol was reviewed and permitted by the institutional research and ethics committee. These patients were diagnosed as having breast cancer by core needle biopsy. The size, number of masses, and location of the tumor were identified through mammography, US, and breast MRI in addition to clinical examination before surgery. The molecular phenotypes were categorized according to estrogen receptors, progesterone receptors, human epidermal receptors (HER2), and proliferation marker (Ki67). A multidisciplinary team, Breast Unit, Cairo University Hospitals, determined that the patients were suitable candidates for NACT. The axillary LNs were considered cN+ according to clinical examination (firm/hard LNs, and fixed or matted by palpation) and by US (completely hypoechoic node with no hilum, focal or diffuse hypoechoic lobulation of the cortex, rounded appearance, or diffuse cortical thickening with a ratio of cortex thickness/hilum thickness >1). Before starting NACT, patients were randomly assigned into two groups: group A, which received TAD, and group B, which received SLNB alone. To confirm CN+ instances in the TAD group, ultrasound-guided FNAC was done from the most suspect axillary node, followed by an injection of 0.2–0.3 ml of carbon particles (Blackeye) under US guidance into the cortex of the sampled node (Fig. 1). NACT (anthracycline/laxane-based regimens) was given to the patients. Anti-HER2 targeted therapy was also given to patients with HER2+ tumors (Trastuzumab). Tumor response to NACT was defined as a complete or partial response (CR and PR), whereas nonresponse was defined as stable or progressing disease in accordance with the Response Evaluation Criteria in Solid Tumors ver. 1.1. (SD, PD). The elimination of metastatic ALNs or the absence of any concerning axilla imaging results in the US was used to characterize the ALN response to NACT. A total of 16 participants were disqualified from this study because they still had axillary illness. We had 40 evaluable patients, with 20 patients in each group. After eliminating patients who did not convert to N0 3–5 weeks after the NACT was finished, all patients had final surgery. Then, 1%MB was injected retroareolarly 15 min before skin incision for SLNB. The axillary fascia was opened at the time of surgery, and stained LNs were looked for beginning at the junction of lateral thoracic vessels and second intercostobrachial nerve. Aside from the SLNB, TAD in group A entailed identifying and removing the tattooed LNs; any blue-stained LNs, larger LNs, or LNs with surrounding blue-stained lymphatic streaks were removed. SLNB was carried out on group B. Both groups have ALND as a backup. LNs were examined by permanent hematoxylin and eosin stains for pathological evaluation. Immunohistochemistry was not routinely used to detect LN metastasis. All slides were also evaluated by an experienced pathologist for metastatic deposits and to ascertain

the presence of tattoo pigment within the cortical surface of the node in the TAD cohort (Fig. 2). Final pathology results of ALND were compared with TAD or SLNB according to the cohort studied. The study protocol was approved by the Faculty of Medicine Cairo University Council.

Results

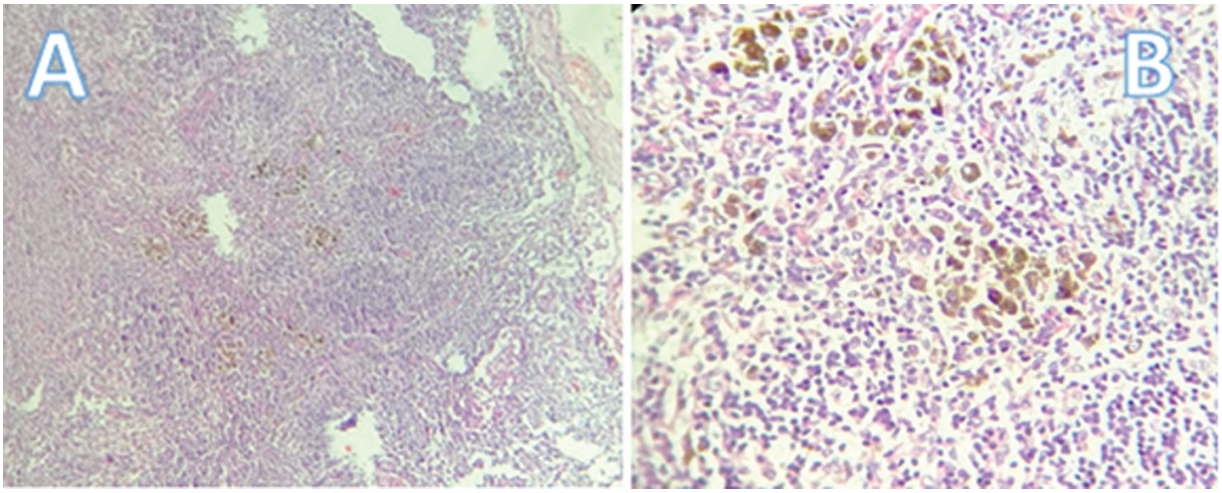
All collected data were revised for competences and logical consistency. Precoded data were entered into the Statistical Package for the Social Sciences (SPSS) version 24.0 (IBM, SPSS Inc., Delaware, Chicago, USA). Baseline features of the patients were reported as means±SD in case of continuous variable or as absolute numbers and percentages in case of categorical data. Categorical variables were compared using the χ^2-test, whereas continuous variables were compared using Student's *t*-test, one-way analysis of variance, and Fisher's exact test where appropriate. Comparison between groups was performed using Student's *t*-test test for quantitative variables and χ^2-test for qualitative variables. Pearson's correlation was applied. For each test, a *P* value of less than 0.05 was considered statistically significant. Patient and tumor characteristics were as follows: the mean age of the patients was 51.2±10.7 years (range: 30–72 years) at diagnosis. The mean size of the main primary tumor at presentation was 6.38±1.8 cm (range: 4–10 cm). The mean LN size at initial presentation was 27.2×18.2 mm (range: 15×6–48×38 mm). The mean LN cortical thickness at presentation was 8.13 mm (range: 4–16 mm). Tumor details are shown in Table 1. None of the patients had complications related to

Figure 1



Axillary ultrasound of a 43-year-old woman with Locally Advanced Breast Cancer (LABC). (a) Before Blackeye ink injection. (b) After Blackeye ink injection in the lymph node cortex (blue arrow).

Figure 2



(a) Section in a lymph node free of metastatic deposits showing dark brown Blackeye ink, hematoxylin and eosin stained (×40). (b) Blackeye ink in a lymph node at higher magnification (×100).

LN Blackeye ink injection (OMNIMED LIMITED Winchester, Hampshire, Engeland) before NACT or to MB injection during or after surgery.

A false-negative (FN) event was defined as a case where the specified LN, either the tattooed/SLNs in group A or SLNs in group B, did not show metastasis even though residual disease was seen in the backup ALND. The FNR was calculated as the number of FN events divided by the total number of pathologically node-positive patients. Negative predictive value (NPV) was defined as the probability that the backup ALND will be negative if the TAD or SLNB (depending on the cohort) is negative. NPV was calculated by dividing true negative (TN) findings by TN plus FN findings. True positive (TP) findings were divided by TP plus FN findings to determine sensitivity. Accuracy was defined as the overall probability that the patient will be correctly classified (the proportion of patients with TP or TN) (Table 2). In group A (TAD), the mean delay between the time of injection and the time of surgery performed was 172.15 days (range: 101–199 days). The Blackeye ink was identified as black stain on the LN in 19/20 cases (95.0%). In one case, no stain on the surface of LN but pathological examination revealed extensive presence of black pigment in the cortex of 'sentinel/no black pigment' LN. Methylene blue 1% was identified successfully in 16 patients (80%) of this group, whereas inked LN was retrieved as an SLN in 87.5% (14 of 16) of the patients (Fig. 3). The mean number of LNs retrieved in this group was 6.1 nodes (range: 4–8). The mean number of LNs in ALND in this group was 10 nodes (range: 8–15 nodes). A total of 11 patients had pathological nodes in TAD/ALND or in TAD specimen. The positive LN in group A was represented by the inked LN alone in 18.2% (2 of 11) of the patients and represented by both ink and MB in 81.8% (9 of 11) of the patients. One of those patient (cN1 before NACT) had a false-negative TAD, resulting in an FNR of 8.3% (P=0.03) (Table 3). In group B (SLNB), the mean number of LNs retrieved was 3.45 nodes (range: 1–6 nodes), (Fig. 4). However, the mean number of ALND nodes was 9.95 (range: 7–14). FNR of SLNB alone was 15.3% as two patients out of 13 had metastatic deposits in the backup ALND but not in the SNLs (P=0.01). In both patients, only two LNs were dissected during the SLNB procedure. However, when three or more SLNs were retrieved, the FNR was 0%. The rest of the data are illustrated in Table 4. When we compared the results of TAD with SLNB, we found that the diagnostic performance of TAD showed the highest values (Table 5).

Table 1 Tumor characteristics at initial presentation and following neoadjuvant chemotherapy

Variable	*N* (%)
Clinical T stage	
T2	7 (17.5)
T3	19 (47.5)
T4	14 (35)
Clinical N stage	
N1	31 (77.5)
N2	9 (22.5)
Tumor biopsy results	
IDC	37 (92.5)
ILC	3 (7.5)
Molecular subtype	
Her2 enriched	4 (10)
Luminal A	2 (5)
Luminal B1	8 (20)
Luminal B2	20 (50)
TNBC	6 (15)
Clinical response	
CR	8 (20)
PR	31 (77.5)
SD	1 (2.5)
Nodal pathological complete response	
Yes	15 (37.5)
No	25 (62.5)

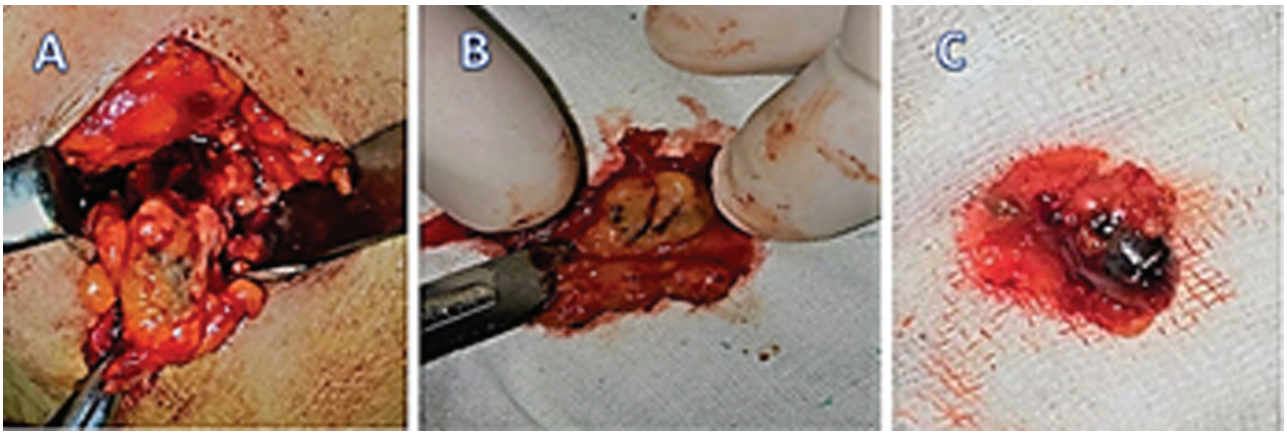
CR, complete response; Her2, human epidermal receptor 2; IDC, invasive duct carcinoma; ILC, invasive lobular carcinoma; N, lymph node; PR, partial response; SD, stationary disease; TNBC, triple-negative breast cancer; T, tumor.

Table 2 Statistical analysis for the study groups

		Residual positive nodes after NAC by final pathology			
1. TAD Group A	Present	*N*	Absent	*N*	Total
Positive	True positive	11	False positive	0	11
Negative	False negative	1	True negative	8	9
Total	12	8	20		
		Residual positive nodes after NAC by final pathology			
2. SLNB Group B	Present	*N*	Absent	*N*	Total
Positive	True positive	11	False positive	0	11
Negative	False negative	2	True negative	7	9
Total	13	7	20		

SLNB, sentinel lymph node biopsy; TAD, targeted axillary dissection.

Figure 3



(a) Identification of black-tattooed lymph node (LN) intraoperative. (b) Cut section in the tattooed LN showing carbon particles. (c) Tattooed node retrieved as a blue sentinel LN in one of the patients.

Table 3 Results of targeted axillary dissection surgery, TAD (group A)

Variable	*N* (%)
Ink identification	
Yes	19 (95.0)
No	1 (5.0)
MB identification	
Yes	16 (80)
No	4 (20)
Tattooed node retrieved as an SLN (i.e. concordance)	
Yes	14 (87.5)
No	2 (12.5)
Positive nodes in ALND	
Yes	6 (30)
No	14 (70)
Positive nodes in TAD	
Yes	11 (55)
No	9 (45)
FNR	1 (8.3)

ALND, axillary lymph node dissection; FNR, false-negative rate; MB, methylene blue; SLN, sentinel lymph node.

Table 4 Results of sentinel lymph node biopsy surgery, SLNB (group B)

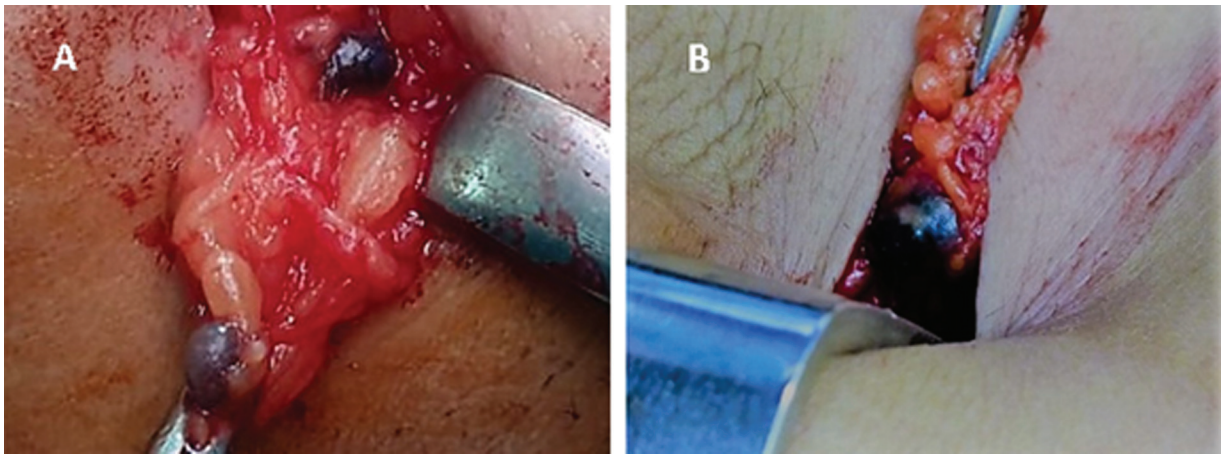
Variable	*N* (%)
MB identification	
Yes	17 (85.0)
No	3 (15.0)
Positive nodes in ALND	
Yes	8 (40)
No	12 (60)
Positive nodes in SLNB	
Yes	11 (55)
No	9 (45)
FNR	
2 or less LNs	2 (15.3)
3 or more LNs	0

ALND, axillary lymph node dissection; FNR, false-negative rate; MB, methylene blue; SLN, sentinel lymph node.

Discussion

The magnitude of the surgical intervention required is highly reliable on the patients' extent of response to NACT; in the axilla, similar efforts to individualize treatment are currently ongoing, and the optimal axillary staging procedure for post-NACT cN0 in patients, who presented with cN+, remains questionable. The ALND clearance is accompanied by unacceptable rates of morbidity despite being the most accurate approach. Initial reports addressing the utility of SLNB after NACT reported FNRs ranging from 5 to 20% [11]. However, studies showed that SLNB was accurate if greater than or equal to three SLNs were removed, using IHC for SLNs examination and combining removal of the marked pretreatment positive node. The idea that the particular node that had been shown to contain metastases by needle biopsy at diagnosis before NACT is the optimal node to measure response after NACT has been previously investigated [8]. Following this, well-planned prospective trials were conducted in which positive LNs that were marked before NACT were removed after NACT with SLNB (TAD procedure) and a dramatic decrease in FNR was observed. To label positive nodes before NACT and to recognize marked nodes after NACT completion, a variety of techniques have been suggested. In the study by Diego and colleagues in 2016 on 30 patients, positive LNs were tagged with a tissue marker (clip) before NACT with ultrasound guidance. In 29 patients after NACT completion the marked nodes were successfully localized using an I 125 radioactive seed (IR of marked nodes:96.7%). Overall, 91% of marked LNs and SLNs were in agreement [12]. When Caudle *et al.*

Figure 4



Sentinel lymph node (LN) biopsy identification. (a) Two blue-stained LNs by 1% methylene blue. (b) Blue LN stained by MB1% after 15 min of injection.

Table 5 Diagnostic performance of targeted axillary dissection (TAD) versus sentinel lymph node biopsy (SLNB)

Variable	TAD (%)	SLNB alone (%)	*P* value
FNR	8.3	15.3	–
Negative predictive value	90.8	77.9	0.017
Success rate	95.2	86.9	0.32
Accuracy	95	90	0.06
Sensitivity	91.7	84.6	0.048

FNR, false-negative rate.

[8] employed the same technique, 5 of 208 patients showed no clipped nodes in the surgical specimen (IR of marked nodes 97.6%), and 77% of marked LNs appeared as SLNs. In 2015, Donker *et al.* [7] marked positive ALNs in 100 patients before NACT using an upfront radioactive seed I125 and reported IR intraoperatively of 97%. In another research, 91 patients had their positive ALNs clipped before NACT, and after NACT completion, a hook wire with ultrasound guidance was used to locate the clipped LNs; in 97.3% of patients who had SLNB or ALND, marked LNs were effectively removed, a rate significantly higher than 79.4% of the 34 patients who did not have wire localization [13]. Choy *et al.* [10] in 2015 used a carbon suspension (spot tm) for tattooing positive ALNs in 28 patients with 96.4% IR of tattooed ALNs. In the study by Park *et al.* [14] in 2018, in 20 patients before NACT, an activated charcoal suspension (Charcotrace tm, Healthdirect, Haymarket NSW, AUS) was used to mark positive ALNs, and IR was 100%, with 75% agreement between marked LNs and SLNs. Finding the most precise method for mapping axillary positive LNs is still difficult today. The placement of a metallic clip into a positive LN under ultrasound guidance is straightforward; the primary challenge here is identifying and localizing the clipped node following NACT. In a pathologic, swollen, hypochoic LN, a hyperechoic tissue marker might be seen by ultrasonography with ease; however, following NACT completion and LN regression, this can be exceedingly challenging or perhaps impossible. There have been a number of tissue markers with improved ultrasound vision proposed; however, there is no 'ideal one'. In addition, patients experience pain when hook wires are inserted into the axillary cavity to target clipped nodes, particularly when two or more nodes need to be localized. The use of a radioactive seed is more complex, more costly, and subject to legal ramifications if seed planting comes before NACT. In our investigation, clinical and radiological examinations of 40 individuals who had previously been cN+ resulted in their conversion to cN0 were sent via a secondary ALND after either TAD or SLNB. In the TAD cohort, we correctly detected the Blackeye ink in 95.0% (19 out of 20) of the patients, and the mean time between the ink injection and surgery was 172 days, with range from 101 to 199 days. In contrast to the use of radioactive seeds, which is more complex and has ethical and legal concerns, we discovered that axillary LN tattooing is a straightforward procedure that is simple to identity after surgery and less expensive.

Additionally, tattoo ink is long lasting and does not require additional radiological imaging or nuclear medicine treatments to pinpoint it. Choy and colleagues, reported that tattooed ALNs were identified in all patients treated with NACT. Intraoperative pigment was detected up to 211 days

after injection, with an average time interval of 130 days [10]. Park *et al.* [14], reported that the black-tattooed ALN can be detected within a median duration of 6 months, fulfilling the technical feasibility of node tattooing without significant morbidity. The end success rate of surgical retrieval of all marked nodes was 98.6% in a recent trial where every biopsy-proven or worrisome node was marked by charcoal tattoo before NACT, and the IR of all marked nodes was 94.6% [15]. We successfully identified the SLN with 1%MB in the TAD cohort in 80% of the patients; when the blue dye was not identified, we removed palpable suspicious nodes together with the tattooed node during TAD surgery. In the TAD group, the tattooed LNs that were retrieved as SLNs stained by 1%MB were 87.5%. The correlation between SLNs and tattooed LNs is not that reliable, approving that TAD improves the accuracy of SLNB for axillary assessment of these patients. In addition, the pathologically positive LN in this cohort was represented by the inked LN alone in 18.2% of the patients, and represented by both ink and MB in 81.8% of the patients. The black pigment of tattooed LNs sometimes obscures the blue color of MB, but detecting the blue color staining the efferent lymphatic vessels indicates the presence of SLN. Following many trials and errors, we had gained enough experience to be able to distinguish between both blue and black pigments with ease. In the SLNB alone group, the IR of MB was 85.0%. Data reported from the ACOSOGZ1071 trial also indicated that 20% of patients did not have the clipped node retrieved as an SLN, which means a concordance rate of 80% [5]. Moreover, Caudle *et al.* [8], stated that the clipped node might not be identified as an SLN with traditional mapping techniques, as was the case in 23% of their population. Furthermore, Choy *et al.* [10], 2015, reported that LNs that were identified as suspicious and tattooed before treatment was initiated correlated 96.4% with those identified as sentinel nodes at the time of definitive surgery. On the contrary, Park *et al.* [14], found the tattooed LN included in an SLN in only 75% (15 of 20) patients, and in the Greek study, the correspondence between tattooed nodes and SLNs was 75.7% [15]. We consider the value of TAD is to decrease the FNR compared with SLNB alone. It also improves the sensitivity, accuracy, and NPV of the SLNB, thus achieving the balance between surgical morbidity and diagnostic accuracy. In our study, TAD was associated with a lower FNR of 8.3% than SLNB alone, which has an FNR of 15.3%. Removal of the clipped node with the radioactive or blue LN could lower the FNR for SLNB to 6.8% [5]. Caudle *et al.* [8], stated that adding evaluation of the clipped node to evaluation of the SLNs reduced the FNR to 1.4% from the FNR of 10.1% for SLND alone (P=0.03). Furthermore, Park *et al.* [14], reported that when the hot and/or blue SLNs and charcoal-tattooed node were calculated together as the modified SLNB, the diagnostic performance of the modified SLNB showed the highest values as follows: sensitivity, 100%; FNR, 0.0%; NPV, 100%; and accuracy, 100%. The following values were found for the diagnostic performance of TAD in our study: FNR, 8.3%; accuracy, 95%; NPV, 90.8%; and sensitivity, 91.7%. However, SLNB alone revealed the following results: FNR, 15.3%; accuracy, 90%; NPV, 77.9%; and sensitivity, 84.6%. Our study has a relatively small number of patients, which indicates that the results must be interpreted carefully. However, being in concordance with two previously published studies by Choy and colleagues and Park and colleagues, our results indicate that TAD of tattooed ALNs is a feasible procedure with high IRs and low FNRs.

Conclusion

The evaluation of the cytologically proven metastatic LN marked at the time of initial biopsy with Blackeye ink following NACT-SLNB using 1%MB (TAD procedure) is a valuable tool of assessing nodal response after NACT. It has a 95% IR and a low FNR of 8.3%. It could be used in the near future to de-escalate the extent of axillary surgery in initially cN+ patients who were converted to cN0 after NACT, with more confidence that TAD has a higher diagnostic performance than SLNB without significant morbidity and at a very low cost.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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