Evaluation of sentinel lymph node biopsy using 1% methylene blue in node-responsive postneoadjuvant chemotherapy in patients with breast cancer

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Background
Surgical management of the axilla in breast cancer has evolved greatly in the past 20 years. Sentinel lymph node biopsy (SLNB), which was first investigated in the early 1990s, has replaced routine axillary lymph node dissection with its associated greater morbidity in early-stage node-negative patients. However, the role and timing of SLNB, management of the axilla, and technical aspects of the procedure in patients treated with neoadjuvant chemotherapy (NACT) are matters of controversy. Although data on SLNB and NACT are accumulating, the accuracy of SLNB to stage the axilla and the value of SLNB in relation to long-term outcomes remain to be seen.
Objectives
To evaluate the use of 1% methylene blue (MB) as a single agent tracer for the detection of sentinel lymph node after NACT for locally advanced breast cancer with the calculation of the identification rate and false-negative rates (FNRs).
Patients and methods
A total of 31 patients with cT3-4(a-c) cN1-3, M0 breast cancer after NACT who showed downstaging of their nodal status to N0 were injected with 1% MB, retroareolarly or peritumorally, followed by axillary lymph node dissection, and the results were studied to detect the identification rate and FNR.
Results
The median age of the patients was 48 years, with 83.8% of the patients above the age of 40 years. The identification rate was found to be 83%, and a FNR of 11.5% was detected. FNRs were found to be less when more than three sentinel lymph nodes were dissected, with no statistically significant difference relating FNRs to the initial nodal state of the disease.
Conclusion
The use of 1% MB after NACT showed a comparable identification rate and FNRs to other single tracer techniques, yet all single tracer techniques are lesser than ideal in this group of patients.

Keywords:
false-negative rates, identification rate, locally advanced breast cancer, sentinel lymph node biopsy

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Introduction

Breast cancer is the most frequently diagnosed malignancy and the second most common cause of cancer-related death in women worldwide [1]. Accurate staging and proper management of axillary lymph nodes (ALNs) are important for the treatment of breast cancer. Surgical management of the axilla in breast cancer has evolved greatly in the past 20 years. Sentinel lymph node biopsy (SLNB), which was first investigated in the early 1990s, has replaced routine axillary lymph node dissection (ALND) with its associated greater morbidity in early-stage node-negative patients. SLNB provides accurate assessment of nodal status and crucial staging information [2]. In patients with early-stage clinically node-negative breast cancer, SLNB, as compared with ALND, has achieved equivalent locoregional recurrence, disease-free survival, and overall survival results, without the added morbidity of ALND [3].

However, the role and timing of SLNB, management of the axilla, and technical aspects of the procedure in patients treated with neoadjuvant chemotherapy (NACT) are matters of controversy. Although data on SLNB and NACT are accumulating, the accuracy

of SLNB to stage the axilla and the value of SLNB in relation to long-term outcomes remain to be seen [2]. Multiple studies have noted lower reliability and accuracy of SLNB after NACT owing to the high false-negative rates (FNRs). In nodal positive (cN+) cancer, the debate is still ongoing about the role of SLNB compared with ALND [4].

Patients and methods

This study was conducted at Al-Kasr Al-Ainy Hospital, Faculty of Medicine, Cairo University, between August 2017 and February 2019. The study protocol was reviewed and permitted by the institutional research and ethics committee. The study involved 31 patients with clinically node-positive breast cancer receiving NACT. All patients involved in the study were consented before the study.

In our study, we enrolled patients with cT3-4(a-c) cN1-3, M0 breast cancer after NACT who showed downstaging of their nodal status to 'N0' as proven by clinical examination and ultrasonographical assessment. Exclusion criteria were pregnancy, inflammatory breast cancer, metastatic breast cancer, and postmenopausal luminal A cases (hormonal treatment).

Patients presenting to the breast surgery unit or medical oncology department at Al-Kasr Al-Ainy University Hospitals were properly examined. Proper history was taken from the patient in the form of present history, menstrual history, family history of previous breast diseases, history of any chronic diseases, and previous breast surgeries. Proper general and local examination was done; those presenting with suspicious masses, or at high risk of breast malignancy, proceeded to further imaging procedures (mammography, ultrasonography, and MRI). If a suspicious mass is found (BIRADS 4-5), an ultrasound-guided biopsy is taken for histopathological examination. Patients with a histopathological diagnosis of breast cancer underwent further metastatic workup according to the stage of presentation (computed tomography chest and abdomen in locally advanced cases) and immunohistochemical study of the tumor in the form of estrogen receptor, progesterone receptor, Her2neu receptors, and proliferation index (PI) Ki67.

A multidisciplinary team with members from the breast oncology, surgery, radiology, and pathology departments arranged for a weekly meeting for proper consultation about the management of patients. Patients with locally advanced masses and cN+ received NACT, where patients who were categorized as having locally advanced breast cancer (LABC) eligible for BCS were offered to receive NACT with the following rationale: postmenopausal patients other than luminal A and premenopausal luminal A, luminal B1, B2, Her2-enriched, and triple-negative patients received anthracycline/taxane-based regimen, and Her2-positive patients received anti-Her2 targeted therapy in the form of trastuzumab. NACT is then followed by surgery aiming for BCS or a safer and easier mastectomy.

Patients who showed nodal response to NACT (downstaging from cN+ to cN0) proved clinically by examination and radiologically by ultrasonography were scheduled for intraoperative SLNB. Retroareolar or peritumoral injection of 3 ml 1% methylene blue (MB) was used to assess the identification rate of the sentinel lymph node (SLN). A transient time of 15 min was given for the MB to reach the LNs, and then a small incision about 2 cm in length just below the hairline was done. The axillary fascia is then opened and properly dissected until the ALNs are visible (SLNs are usually found at the intersection of lateral thoracic vein and intercostobrachial nerve). Any blue-stained LN, enlarged LN, or LNs with blue-stained lymphatic streaks were excised. A back-up ALND was done in the same setting, and then the results of the SLNB and the ALND were compared to detect false-negative results of the SLN when 1% MB was used. Moreover, the identification rate of SLN with 1% MB was reported.

Results

A total of 31 patients with noninflammatory LABC according to the AJCC staging who received NACT and showed complete nodal response were included in this study. The median age of the patients was 48 years, with 83.8% of the patients above the age of 40 years. Tumor details are analyzed in Tables 1 and 2.

Regarding response in the axilla, seven patients showed complete pathological response in the axilla, with a percentage of 22.5%. However, the overall tumor complete pathological response was found in six patients, with a percentage of 19.4%.

Regarding SLN identification, SLN was successfully identified using 1% MB in 26 patients, with an identification rate of 83% (26/31). Back-up ALND was then done, and the results of LN status in ALND were compared with those of the SLN. One out of the five cases in which SLN was not identified had positive ALN deposits.

SLN was positive in 20 of 26 cases; of the six cases that showed negative SLNs, three cases were concomitant

Table 1 Tumor details

	Count	%
Mass		
T2	5	16.1
T3	10	32.3
T4	16	51.6
LNs		
N1	18	58.1
N2	13	41.9
Pathology		
IDC	25	80.7
ILC	6	19.3
Luminal classification		
A	3	9.7
B1	7	22.6
B2	16	51.6
Basal like	4	12.9
Her2neu enriched	1	3.2

LN, lymph node.

Table 2 Status of axillary lymph nodes retrieved

Number of SLNs		
0	5	16.1
1	3	9.7
2	3	9.7
3	13	41.9
<3	7	22.6
SLN		
Negative	6	19.4
Positive	20	64.5
Failed	5	16.1
ALND		
Positive	24	77.4
Negative	7	22.6

ALND, axillary lymph node dissection; SLN, sentinel lymph node.

with the ALND results, whereas the other three cases were falsely negative, with positive tumor deposits in ALND specimens. Thus, the FNR was 3/26 (11.5%) in the whole group.

A total of 15 patients had an initial N1 LN before NACT. SLN was positive in 10 (66.7%) of them and negative in five (33.3%). Two cases of those who did not show SLN deposits had LN deposits on ALND (false negative). The FNR in N1 patients was 16.6%. Overall, 11 patients had an initial N2 before NACT, of which 10 showed positive SLNs and one case showed false-negative results when compared with ALND, with a FNR of 9.1%. No statistical significance was found between N1 and N2 in the FNRs, with a *P* value of 1.

Less than three LNs were dissected in six patients, of which, three were positive and two were false negatives, with a FNR of 40%. Three or more LNs were dissected in 20 patients, of whom 13 were positive and one was false negative, with a FNR of 7.1% in this subgroup of patients. Statistical significance was found when more than 3 SLNs were dissected, with a *P* value of 0.04. Known complications from MB injection such as allergic reaction, superficial sloughing of the skin, and permanent pigmentation of the skin did not appear in any patient of the studied group.

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences). Statistical analysis was done using IBM SPSS statistics for windows, Version 23.0 (IBM Corp., Armonk, NY, USA). Data were summarized using mean, SD, median, minimum, and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. For comparing categorical data, χ^2 test was performed. Exact test was used instead when the expected frequency is less than 5 [5]. *P* values less than 0.05 were considered as statistically significant (Figs 1 and 2).

Discussion

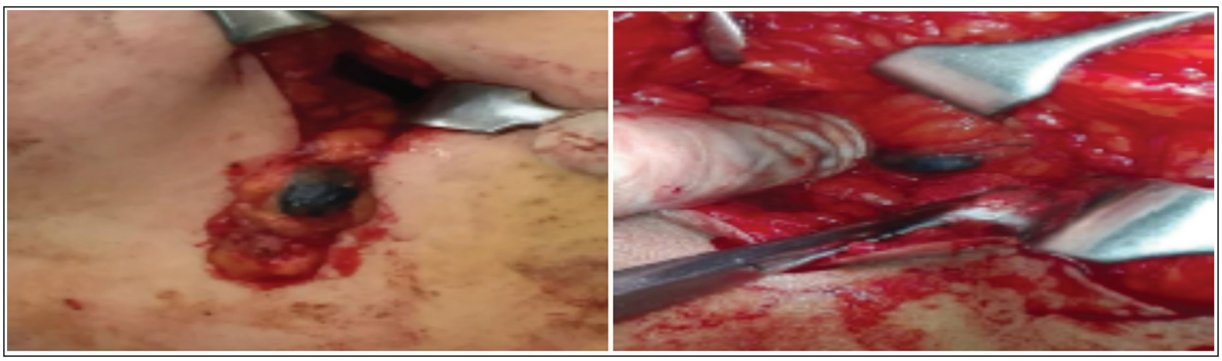
During the past few years, there have been a number of clinical trials on the effectiveness and role of SLNB after NACT. According to their findings, SLNB after NACT seems to be an acceptable procedure, despite varying degrees of false-negative results. However, the reliability of SLNB following NACT for patients with initial nodal disease has been questioned, as the only available data have been from small series, reporting FNRs ranging from 7 to 25%.

Currently, ALND after NACT in patients with proven node-positive disease at presentation is recommended. However, the ALN metastases may have been eradicated by NACT in certain patients, who could consequently be spared ALND. Even in patients with nodal disease at presentation, sparing those patients the morbidity associated with axillary dissection would be desirable [6].

In this study, 31 patients who were previously N(+) received NACT and converted to N(0), by clinical and radiological evaluation, had SLNB, and after the injection of 1% MB, SLNs were successfully identified in 26 patients, with an 83% identification rate. The results were compared with other studies over the past two decades.

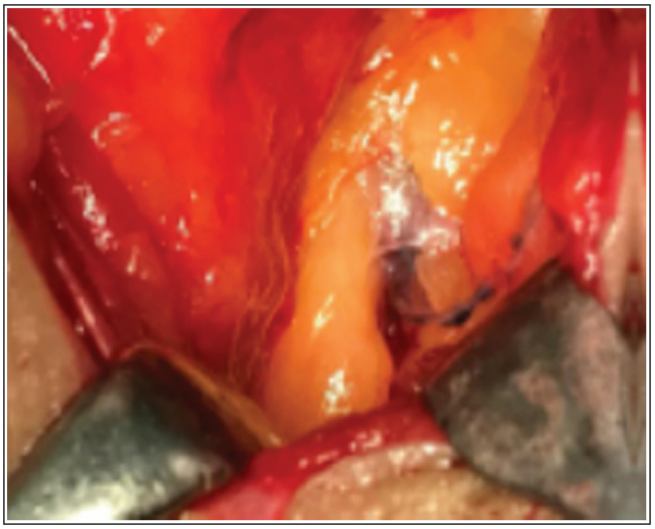
In a study by Kim *et al.* [6], which involved 386 patients who had proven axillary disease, as proved by fine-needle aspiration, over the period between January 2007 and August 2013, of which 266 underwent ALND without SLNB, 120 underwent SLNB followed by ALND. The

Figure 1



SLN identified in two of our cases after 15 min of injection of 1% methylene blue. SLN, sentinel lymph node.

Figure 2



Blue-stained lymphatic streaks identified by 1% methylene blue in one of the cases.

identification rate was found to be ~95.8% (115/120) using a single tracer either blue dye such as isosulfan blue or a radionucleotide.

The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-27 trial is one of the largest studies published to date on SLNB after NACT. A total of 428 patients underwent SLNB with concomitant ALND after NACT with an identification rate of 84.8% using the blue dye isosulfan blue [7].

Compared with other studies, this study showed that 1% MB has an identification rate of 83%. Other studies showed comparable rates while using different detection agents such as isosulfan blue dye. Yet, they were all considered to be insufficient if used alone, and studies showed better results when a dual agent tracer was used in the detection of SLNs. FNRs in our study were found to be 11.5% (3/26) in the 26 patients in which SLNs were successfully identified. No significant difference was found to be related to the initial nodal status whether N1 or N2 in FNRs or identification rates.

The ACOSOG Z1071 trial showed that the FNR of SLNB after NACT in patients with cN1 breast cancer and at least two SLNs identified at the time of surgery was 12.6% higher than the expected threshold of 10% [8]. A strategy in which SLNB was only performed on patients with nodes that appeared normal on axillary ultrasound (AUS) was modeled and projected to potentially reduce the FNR among patients from 12.6 to 9.8%, that is, below the previously prescribed threshold of 10% [8].

FNRs of SLNB in SENTINA patients after NACT were higher than the Z1071 study. In arm C patients with pretreatment cN+ disease that became ycN0, the FNRs of SLNB after chemotherapy were 24.3% for women who had one sentinel node removed and 18.5% for those who had two nodes removed [9].

The results of Kim and colleagues, showed that SLNs that stained blue or were hot, including suspicious nodes, were identified; the FNRs were 10%. The number of SLNs retrieved affected the FNRs.

In this study, 20 patients had three or more SLNs dissected and examined with a FNR of 7.1% compared with 40% when less than 3 SLNs were dissected. The NSABPB-32 trial, in which SLNB surgery was performed before any chemotherapy, reported that there was a significant decrease in the FNR as more SLNs were resected: 18% with 1 SLN resected, 10% with two SLNs resected, and 7% with three SLNs resected [10].

SENTINA showed a FNR of 4.9% when three or more LNs were dissected in contrast to 18.5% when

only two LNs were dissected. Dissection of one SLN in this study showed a FNR of 24.3%.

The Z1071 had also shown better results when three or SLNs were dissected with a FNR of 9.1% compared with a 21.1% when two LNs were dissected. FNRs were even higher when only one SLN was dissected with a 31% rate.

A secondary goal of ACOSOG Z1071 is a determination of AUS accuracy in LABC after completion of NACT. Patients with axillary nodes that appeared suspicious on AUS had more positive nodes and larger nodal metastases compared with patients with sonographically normal appearing nodes [8].

Another method of reducing the FNR of selective axillary surgery after NACT is through targeted axillary dissection of lymph nodes that had previously been found to harbor disease via pretreatment ultrasound-guided FNA and that were marked with a clip at the time of biopsy. In a prospective study at MD Anderson, previously clipped nodes with pretreatment evidence of metastatic disease were localized with radioactive I-125 seeds and removed at the same time as sentinel nodes. The combined excision of both sentinel and seed localized clipped nodes, together yielded a FNR of only 2% [11]. Similar results were reported by Khallaf *et al.* [12], who had performed targeted axillary dissection of carbon-tattooed metastatic lymph nodes in combination with postneoadjuvant SLNB using 1% MB.

Conclusion

The use of 1% MB in post-NACT showed a comparable identification rate and FNRs to other single tracer techniques, yet all single tracer techniques are lesser than ideal in this group of patients. However, if the ALNs were negative by preoperative AUS and three or more SLNs are retrieved, the FNRs improve.

Thus, the use of AUS for better selection of SLNB after NACT has the potential to shift the care of LABC away from the one in which ALND is mandatory for all patients and toward a more individualized approach in which ALND and its concomitant morbidity are safely avoided in selected patients. As for now, however, a complete levels I and II ALND is still recommended for definitive staging and control of axillary disease for patients with LABC treated with NACT [13].

One percent MB is an available, cheap, with low rate of complications compared with other tracers used in SLNB, and its use is recommended to expand the availability of SLNB for patients with breast cancer in our community and its use may extend to post-NACT SLNB.

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Conflicts of interest
There are no conflicts of interest.

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