Assessment of the efficacy and oncological safety of sentinel lymph node biopsy in node-negative breast cancer using methylene blue dye

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Background
Sentinel lymph node (SLN) biopsy in patients with breast cancer with clinically negative axillary nodes is an innovative technique in the management of the axilla. SLN biopsy has been performed using different techniques including injection of patent blue dye, radioactive colloid, and recently methylene blue dye. The aim of this study was to assess the safety and efficacy of methylene blue dye as a mapping agent for SLN biopsy in clinically axillary node-negative breast carcinoma.
Patients and methods
Between January 2014 and October 2016, 50 female patients with established diagnosis of breast carcinoma by tru-cut biopsy and clinically negative ipsilateral axillary lymph nodes were included in the study. All the patients were operated upon in Ain Shams University hospitals. After induction of anesthesia, 3–5 ml of sterile 1% methylene blue was infiltrated into the subareolar tissue on the affected side. The lymph nodes receiving the blue dye were excised as the SLN. Excised specimen with the axillary tissue was sent for histopathological examination. The presence or absence of metastasis in SLN and axillary lymph nodes was compared. Statistical analysis was carried out to know sensitivity, specificity, and accuracy of SLN biopsy in breast cancer.
Results
The incidence of breast cancer was highest at 41–50 years. Of our 50 cases, SLN was identified in 44 cases using methylene blue dye. The identification rate was 88%. None of the patients had negative SLN but positive axillary lymph nodes (false negative), and in six cases, SLNs were involved only but not the rest of the axilla (false positive). The sensitivity, specificity, positive predictive value, and negative predictive value were 100, 85.7, 25, and 100%, respectively.
Conclusion
This study confirms the safety and efficacy of methylene blue dye as a mapping agent for SLN biopsy in axillary node-negative breast cancer.

Keywords:
breast cancer, methylene blue dye, sentinel lymph node biopsy

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Introduction

Breast carcinoma is the most common cancer of women worldwide, including 23% of all female cancers [1]. Approximately one in nine women will have breast cancer during her lifetime. Breast cancer is the commonest cause of deaths owing to cancer in females throughout the world [2].

The incidence of breast cancer is increasing. This increase in incidence may be because of screening, self-examination, and awareness. Staging is very important for management of all patients with cancer, and breast cancer is not an exception. Staging of axilla in breast cancer is the single most important prognostic factor for selection of appropriate adjuvant therapy, locoregional recurrence, and long-term survival. Exact staging of axillary lymph nodes can be obtained in two ways: directly by axillary lymph node dissection (ALND) or indirectly by sentinel lymph node biopsy (SLNB). The ALND is drastic and associated with debilitating complications of the ipsilateral arm like lymphedema, seroma, paresthesia, etc.; whereas, SLNB is less drastic and devoid of aforementioned complications [3–5].

SLN biopsy by radio-colloid method was first reported by Krag *et al.* [6] and by blue dye method by Giuliano *et al.* [7]. Combined use of radioactive colloid and blue dye injection is considered as gold standard for axillary SLNB in breast cancer, with 97% accuracy rate [8–10], but this combined usage does not attain an adequately

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higher detection rate to defend the cost [11]. However, some researchers have been using blue dye only for identification of SLN with good reliability [12]. The positive results found by using methylene blue dye and by isosulfan blue dye were 99 and 97%, respectively [13,14]. Moreover, another similar study for methylene blue dye was done, showing the sensitivity and specificity of 85.7 and 71.4%, respectively [15]. Therefore, the efficiency of detecting SLN by methylene blue is as good as isosulfan blue with cost-effectiveness and is equal to ALND in breast cancer, but there is difference between the percentages of positive results in different studies [15].

For adopting SLNB technique, it is a well-recognized accepted fact that a multidisciplinary team, which includes surgery, nuclear medicine, and surgical pathology departments, is required to work in close cooperation. Each of these disciplines plays a crucial role in achieving success, and the surgeon cannot embark upon a successful SLNB program without cooperation from other disciplines.

In this study, we had chosen the subareolar technique in detection of sentinel lymph nodes (SLNs) using methylene blue dye only, together with the assessment of its accuracy, efficacy, and oncological safety in clinically node-negative patients.

Patients and methods

Between January 2014 and October 2016, a series of 50 consecutive female patients presenting with breast cancer proven with true-cut biopsy, and clinically node-negative axilla, were included in this prospective study. Patients with palpable axillary lymph nodes, distant metastasis, previous breast cancer surgery, neoadjuvant treatment, inflammatory breast cancer, pregnant females, male patients, and patients unwilling to participate in the study were excluded. Local ethical committee approval was given for the study, and written informed consent was obtained from all participants. This study was performed in Ain Shams University hospitals in Cairo, Egypt.

All patients underwent bilateral sono-mammographic examination, routine preoperative investigation, (complete blood count, prothrombin time and concentration, renal function test, and liver function test), and ECG for assessment for surgical fitness. Metastatic workup was done, including chest radio graphy, abdomen and pelvis ultrasonography, and isotope bone scan, to exclude presence of distant metastasis.

The procedure is carried out under general anesthesia with the patient supine on the operating table and the arm abducted at 90° from the body. After draping, 5 ml of 1% methylene blue dye was injected in the subareolar region, divided in three injections times (Fig. 1). Massage was done for 10–15 min in a clockwise direction. A useful anatomical landmark is made to place the incision 1 cm below the hairline of the axilla. Skin and subcutaneous tissue were dissected followed by dissection of clavipectoral fascia to enter the axilla, and blue-stained lymphatics were identified. Following the stained lymphatics, identification of the blue colored node(s) is made, which presented mainly below the pectoralis minor muscle (Fig. 2). These nodes, together with perilymphatic tissue, were dissected and were labeled separately, and then, planned procedure, either modified radical mastectomy (MRM) (Fig. 3) or wide local excision (WLE), was performed. The breast tissue or excised mass along with the remaining axillary lymph nodes were histopathologically examined separately from sentinel lymph nodes. The presence or absence of metastasis in SLN and axillary lymph nodes was compared. Statistical analysis was carried out to know sensitivity, specificity, and accuracy of SLN.

Results

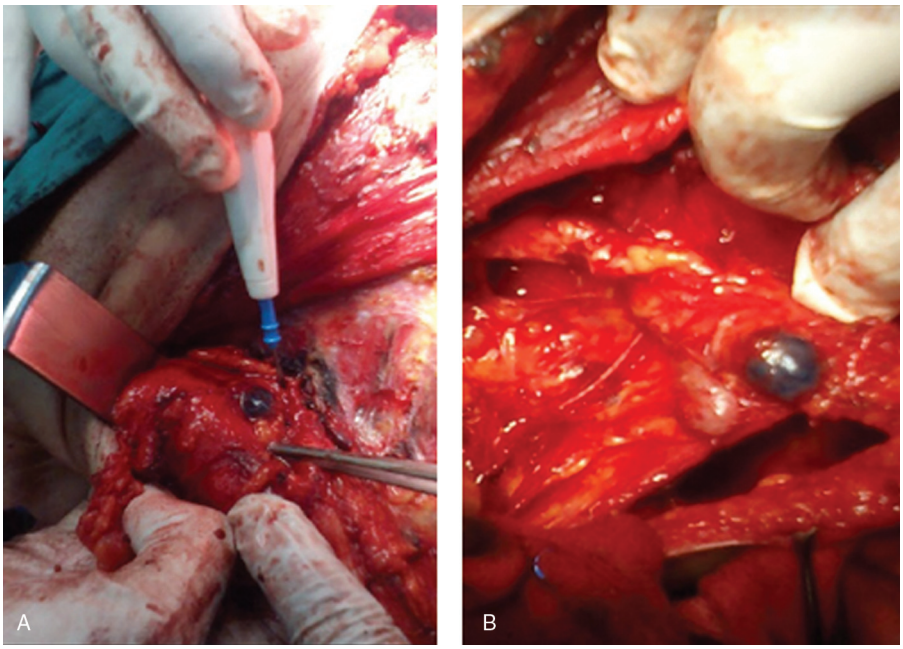
Age of the study group

The age of the 50 female patients included in the study ranged between 30 and 70 years, with a mean age of 45.7±1.0 years. A total of 13 (26%) patients were between the age of 30 and 40 years. Patients between the ages of 41 and 50 years represented the highest percentage with 18 (36%) cases, followed by patients between the age of 51 and 60 years, with 12 (24%) cases, and finally, those older than 60 years, with seven (14%) cases (Table 1 and Fig. 4).

Figure 1

Injection of methylene blue dye in the subareolar region.

Figure 2

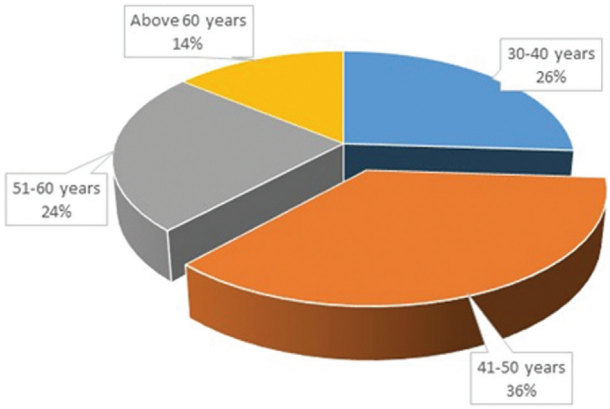


(a,b) Identification and dissection of sentinel lymph node in the axilla stained with methylene blue dye.

Figure 3

Modified radical mastectomy specimen together with remaining axillary specimen after dissection of sentinel lymph node.

Figure 4



Age of the study group.

Table 1 Age of the study group

Age groups	*n* (%)
30–40 years	13 (26)
41–50 years	18 (36)
51–60 years	12 (24)
>60 years	7 (14)

Table 2 Stage of tumor among the study group

Size of the tumor	*n* (%)
<2 cm (stage I) (T1, N0, M0)	3 (6)
>2–4 cm (stage IIA) (T2, N0, M0)	38 (76)
>4–5.5 cm (stage IIB) (T3, N0, M0)	9 (18)

Size and stage of the tumor

In our study, the size of the tumor ranged between 1.5 and 5.5 cm, with a mean size of 3.4 cm. A total of 38 (76%) patients had tumor size ranging from 2 to 4 cm (T2N0M0) and nine (18%) patients had tumor size ranging from 4 to 5.5 cm (T3N0M0), and finally, three (6%) patients presented with tumor less than 2 cm (T1N0M0). None of the patients had palpable axillary lymph nodes or distant metastasis (Table 2 and Fig. 5).

Site of the tumor

The outer upper quadrant was the most common site in 29 (58%) patients, the lower outer quadrant was involved in 14 (28%) patients, the upper inner quadrant in five (10%) patients, and central lesion in

two (4%) patients (Table 3). A total of 30 (60%) patients presented with left-sided breast cancer, and the rest (20 patients) presented with right-sided breast cancer (40%) (Fig. 6).

Detection of sentinel lymph node(s)

The detected SLNs were identified in 44 (88%) of 50 patients. The number of SLNs was as follows: one lymph node detected in 16 (32%) patients, two lymph nodes in 19 (38%) patients, and three lymph nodes in nine (18%) patients (Table 4).

In 47 (94%) patients, SLN biopsies were found in level I alone, and in three (6%) patients, SLN biopsies were found in both levels I and II.

Comparison between axillary and sentinel lymph nodes

When status of axillary lymph node was compared with SLNs, the result was as follow: 36 (81.8%) of the 44 patients with identified SLN showed negative result for metastasis. In this group, we found that none of them showed axillary metastasis.

In eight (18.2%) of the 44 SLNs cases, metastasis was found in the nodes. Of these eight cases, two (25%) cases contained metastasis in the remainder axillary lymph nodes whereas in the other six (75%) cases, the results of the axillary lymph nodes were negative (Table 5).

Table 3 Site of the tumor among the study group

Site of the tumor	*n* (%)
Outer upper quadrant	29 (58)
Outer lower quadrant	14 (28)
Inner upper quadrant	5 (10)
Central lesion	2 (4)

Table 4 Sentinel lymph nodes detected among the study group

Sentinel LN	*n* (%)
1	16 (32)
2	19 (38)
3	9 (18)
Negative	6 (12)

The relation between axillary and SLNs is statistically significant (Fig. 7).

Result of lymph node localization

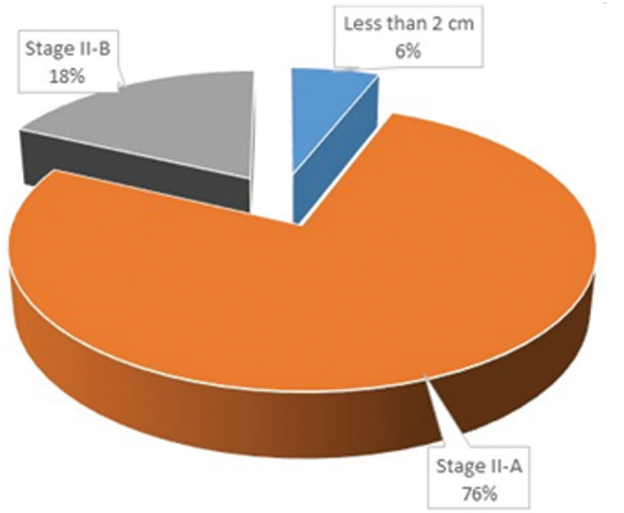
The identification rate for lymph node localization was 44/50 (88%).

Failure rate of the technique was 6/50 (12%).

Sensitivity statistics

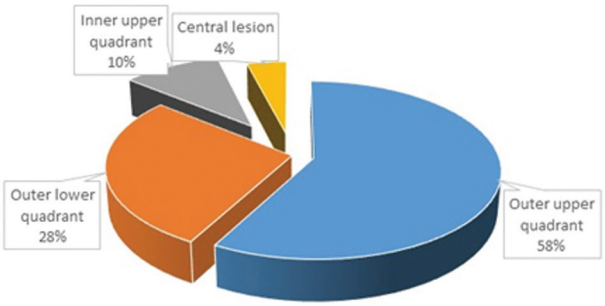
The number of true-positive cases (positive sentinel and positive axillary) was 2.

Figure 5



Stage of tumor among the study group.

Figure 6



Site of the tumor among the study group.

Table 5 Axillary and sentinel lymph node metastasis

Sentinel LN	Axillary metastasis [*n* (%)]	No axillary metastasis [*n* (%)]	χ^2	*P* value
Present sentinel LN				
Positive metastasis	2 (25)	6 (75)	11.44	0.003 (S)
Negative metastasis	0	36 (100)		
Absent sentinel LN	0	8 (100)		

The number of true-negative cases (negative sentinel and negative axillary) was 36.
The number of false-positive cases (positive sentinel and negative axillary) was 6.
The number of false-negative cases (negative sentinel and positive axillary) was 0.
Sensitivity=2/(2+0)=100%.
Specificity=36/(36+6)=85.7%.
Positive predictive value=2/(2+6)=25%.
Negative predictive value=36/(36+0)=100%.
Accuracy=(sensitivity+specifitiy)/2=92.9%.

Operation done for the patients

Overall, 16 (32%) patients underwent MRM, whereas 34 (68%) underwent wide local excision.

Histological type

Histological type showed that 47 (94%) patients had invasive ductal carcinoma, whereas three (6%) patients had invasive lobular carcinoma.

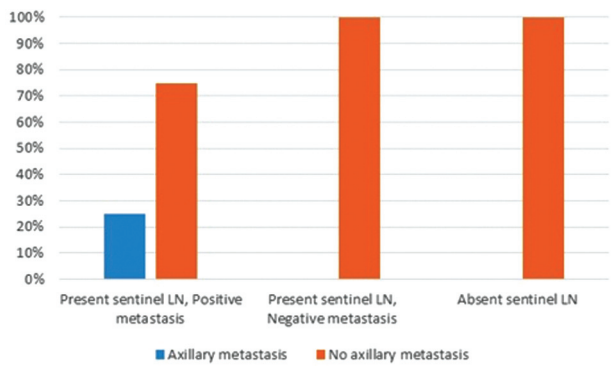
Postoperative morbidity

Postoperative morbidity results showed that three (6%) patients developed seroma, one (2%) patient developed hematoma, and one (2%) patient developed infection and necrosis. The remaining 45 (90%) patients had no postoperative complications. No complications related to the use of the dye, such as cutaneous and urine staining or allergic reactions, occurred in any of our patients (Table 6).

Discussion

Management and staging of breast cancer according to axillary nodal status has been the subject of intense debate and controversy [16]. The study of NSABP B-04, which randomized patients with clinically uninvolved axillary nodes to radical mastectomy, total mastectomy plus radiotherapy, or total mastectomy alone, demonstrated that axillary treatment with either dissection or regional radiotherapy reduced axillary recurrence rates from 18.6 to 1–2%. However, there was no benefit to axillary treatment in terms of distant disease-free survival [16,17].

Figure 7



Axillary and sentinel lymph node metastasis.

Sentinel lymph node dissection (SLND) alone is widely accepted as an axillary management for women with clinically node-negative breast cancer. SLND makes axillary procedure more conservative, less morbid, and improves the quality of life, with reduction in pain, lymphedema, and shoulder stiffness [18].

In this study, we conducted a validation study on the accuracy of SLND using methylene blue dye technique alone in patients with nodal negative breast cancer, using a simple, available, and cheap technique.

In our study, we did not expose our patients to oncological risk because we completed ALND after detection of SLN.

In this study, we excluded patients who received neoadjuvant chemotherapy because this produces an inflammatory response and fibrosis. Therefore, it is not surprising that identification and dissection of sentinel nodes is a more difficult procedure after neoadjuvant chemotherapy [19], and the sentinel lymph node biopsy identification rate is too low for routine use, and that the false-negative rate is also too high [20].

By contrast, there are those who believe that the advantages to the patient in reducing an unnecessary axillary clearance is such that SLNB has a definite role after neoadjuvant chemotherapy [21].

Moreover, we excluded node-positive patients (clinically and radiologically) because positive nodes may be blocked, and it prohibits accurate mapping leading to a false-negative result. This agrees with the studies performed by Lyman *et al.* [22] and Hoar and Stonelake [23].

In contrast, some authors report that clinically positive axilla is subject to false-positive result, so SLNB deserves wider consideration as an alternative to ALND in clinically positive patients [24,25].

Table 6 Postoperative morbidity

Postoperative	*n* (%)
Seroma	3 (6)
Hematoma	1 (2)
Infection and necrosis	1 (2)
None	45 (90)

Inflammatory breast cancer was also excluded. The false-negative rate for patients with inflammatory breast cancer is unacceptably high, hence SLNB is not recommended in such situation until more data are available [26].

Pregnant women with breast cancer were excluded because vital dyes should not be administered to pregnant women [26].

In our study, we excluded male breast cancer cases because of the rarity and no sufficient previous studies about drawback of SLNB in breast cancer in men. However, there are studies that encourage SLNB in men [27].

In our study, we found that among the series of 50 patients, the number of patients between ages of 30 and 40 years was 13 (26%), the number of patients between age of 41–50 years – which was the highest – was 18 (36%), the number of patients between the age of 51–60 years was 12 (24%), and finally, the number of patients older than 60 years was seven (14%). This was a similar figure to the study performed by Mahadevan *et al*. [28], where the highest number of breast cancer cases fell in the age group of 41–50 years (33.1%), followed by 31–40 years (27.8%) and 51–60 years (20.4%).

In our study, 38 (76%) patients were staged as IIA (T2 N0 M0) with a tumor size of 2–4 cm, and nine (18%) patients as stage IIB (T3 N0 M0) with a tumor size of 4–5.5 cm, and finally three (6%) patients as stage I (T1 N0 M0) with a tumor size less than 2 cm. This pattern coincides with the pattern in the study by Ravichandran *et al.* [29] where 151 patients had stage I, 315 patients had stage II, and excluded patients with higher stages of breast cancer.

We found that the outer upper quadrant was the most common site in 29 (58%) patients, the lower outer quadrant in 14 (28%) patients, the upper inner quadrant in five (10%) patients, and central lesion in two (4%) patients. A total of 30 patients presented with left-sided breast cancer (60%), and the rest (20 patients) presented with right-sided breast cancer (40%).

Moreover, Wilting and Hagedorn reported in their study that left-oriented breast cancer (especially upper outer quadrant) showed 45.8% positivity, having a 10% lead over the right orientation, and this was in consonance with the report by Tulinius and colleagues. Wilting and Hagedorn showed that the left side of the body is prone to carcinomas, especially breast cancer (5–10%) [30,31].

Regarding the site of the dye injection, we favored the subareolar injection, as it can access the subareolar lymphatic plexus of Sappey, drainage is independent of tumor size, requires less amount of the dye, and increases identification rate as compared with other methods of injection as demonstrated by McMasters *et al.* [32], D'Eredita *et al.* [33], and Povoski *et al.* [34].

In our study, SLNs were identified in 44 (88%) patients. The number of SLNs was as follows: one lymph node detected in 16 (32%) patients, two lymph nodes in 19 (38%) patients, and three lymph nodes in nine (18%) patients. The mean number of identified lymph nodes in our study was 1.6, and this finding matched with other studies such as Montumora *et al*. [35], Cox *et al*. [36], and Padmanabh *et al*. [37], where the mean number of identified lymph nodes was 1.8, 1.9, and 1.6, respectively.

In 47 (94%) patients, SLN biopsies were found in level I alone, and in three (6%) patients, SLN biopsies were found in both levels I and II. This matches with SLN report by Arima *et al*. [38], who postulated that axillary nodes with breast cancer have a relatively low rate of involvement of level II or level III nodes in the absence of involved level I nodes (called skip metastases), and there is a 2–4% rate of skip metastases above axillary levels I and II.

In our study, we did not find skip metastasis mostly owing to small number of patients (50) and detected SLN in 39 patients. This matches with an Indian study done by Padmanabh *et al*. [37], in which the number of patients was 35.

Overall, 34 (68%) of our patients underwent WLE and ALND, whereas 16 (32%) underwent modified radical mastectomy. Most patients underwent MRM upon their wishes, and two patients had multicentric lesions and two patients showed multiple positive margins after WLE.

Time needed for WLE ranged from 65 to 90 min, including frozen section of the mass and SLN examinations. On the contrary, time needed for MRM ranged from 60 to 105 min.

Drains after surgery were left for 48–72 h for observation of reactionary hemorrhage. The mean

amount of seroma in the first day was 250 cm^3, second day was 150 cm^3, and third day was 50 cm^3.

Postoperative morbidity showed that three (6%) patients developed seroma. These patients were treated conservatively by continuous aspiration under US guidance and coverage of antibiotics. Seroma subsided in 10–15 days. Moreover, one (2%) patient developed hematoma. This patient was treated conservatively, and hematoma subsided within 13 days. Another patient (2%) developed infection and necrosis. This patient underwent debridement of necrotic tissue after 1 week of conservative management, and the wound healed within 10 days. The remaining 45 (90%) patients had no postoperative complications.

The technique of SLNB, as any surgical manoeuver, is not devoid of complications related to technique as mentioned before or complications related to dye such as cutaneous staining, staining of urine, and allergic reaction, which was not reported in any case in our study.

Histological examination showed that 47 (94%) patients had invasive ductal carcinoma, whereas three (6%) patients had invasive lobular carcinoma. This was in agreement with Vahdaninia and Montazeri [40] who found that more than three-quarters (77.5%) of patients were diagnosed with ductal carcinoma and 41.7% of tumors were moderately and well-differentiated.

When we compared status of axillary lymph node with SLNs, the result was as follow: 36 (81.8%) patients of the 44 patients with identified SLN were negative for metastasis, and none of them showed axillary metastasis. In the rest of eight (18.2%) cases, metastasis was found. Of these eight cases, two cases contained metastasis in the remainder axillary lymph nodes (25%) whereas in the other six cases, the axillary lymph nodes were negative (75%). Moreover, Fraile *et al*. [41] reported that 1–15% of patients with negative SLNB had nodal metastasis in the same region, and the false-negative rate of SLNB has improved over time and is probably under 5% now in most experienced group [42].

In our study, SLNs were not identified in six cases, with a failure rate of 12%. This failure corresponded to many factors as following:

First, there is a well-documented learning curve of operator to SLNB. Successful identification of SLNB is directly related to surgeon experience [19]. In this study, we cannot identify 5/6 in the first 25 cases with percentage of 83.3% of failed cases. To decrease the failure rate that related to learning curve, we should take into consideration the multidisciplinary approach between the surgeon, the radiologist, the pathologist, and nursing acquiring the knowledge and skills to enable successful technique. In UK, a structured training programme called NEW START has been developed to standardize technique. This programme includes training of surgeon on five cases followed by performing a series of 25 cases of SLNB and immediate ALND. The aim is to identify SLNB with high rate and more importantly a low false-negative rate [19]. The American Society of Breast Surgeons [43] recommends that surgeons must perform at least 20 SLNB procedures before doing it therapeutically.Second cause is a metastatic lymph node causing a blockage to the lymphatic flow, which was found in four (66.6%) of six cases. To avoid this, careful palpation and an efficient ultrasonic examination should be provided [44].

Third cause is the age of the cases. We found that all six cases were older than 55 years [39].

The technique of SLNB can be applied in most of Egyptian hospitals that could not provide the supplies for SLNB mapping using Tc99m dye and gamma camera as a safe, cheap, reliable, and cost-effectiveness technique.

In our study, SLN was identified in 44 cases using methylene blue dye. The identification rate was 88%. None of the patients had negative SLN but had positive axillary lymph nodes (false negative), and in six cases SLN were involved only but not the rest of the axilla (false positive). The sensitivity, specificity, positive predictive value, and negative predictive value were 100, 85.7, 25, and 100%, respectively, and the overall efficacy was 92.9%.

These results should be compared with the study done by Mukherr and colleagues in 2014, where the identification rate was 88.9%. The sensitivity, specificity, positive predictive value, negative predictive value, and efficacy were 81.8, 100, 100,86, and 90.9%, respectively [45].

Our results are also comparable with the study performed by Chintamani *et al.* [46] where SLN identification rate was 100%, the sensitivity of SLNB was 86.6%, and the accuracy was 93.3%.

Our results showed that the technique of SLNB using methylene blue dye alone is reliable to detect the state of axillary lymph node, so we can avoid an unnecessary lymph node dissection in nodal negative breast cancer and its associated complications.

The technique of SLNB preserves a functioning limb, especially in developing countries as Egypt, where the women in rural area do their work manually.

Conclusion and recommendations

SLNB using methylene blue dye is a suitable, cheap, safe, and accurate technique in staging of the axilla and an alternative to in early stages of breast cancer. Moreover, it is associated with less morbidity.

Our breast surgeons should be trained on this simple technique to achieve high accuracy and lower false negative rate, and our institutes involved in breast cancer surgery should encourage this method because of its advantages regarding safety, feasibility, and economic advantages.

There are still many unanswered questions about SLND that should be answered in on-going trials.

(1) The first one is accuracy of SLNB in large and multifocal tumors.
(2) The second one is accuracy of SLNB after neoadjuvant chemotherapy.
(3) Role of SLNB in nodal positive patients.
(4) Overall disease-free survival.
(5) Role of SLNB in recurrent breast cancer and in male patients.

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Conflicts of interest
None declared.

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