Evaluation of the Efficacy of Combined Sentinel Lymph Node Biopsy Using Patent Blue Dye and Peripheral Lymph Node Biopsy in Management of Nodal Negative Early Breast Cancer

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Introduction: Sentinel node biopsy is now established as the standard care for women with breast cancer. Its accuracy has been validated through multiple clinical trials but with less morbidities than ALND such as pain, restriction of arm movement and lymphedema.

Aim of work: To assess the efficacy of combined SLNB using patent blue dye and PLN sampling in the management of nodal negative early breast cancer. We aimed to avoid the skip metastases phenomenon that occurs with SLN biopsy and to avoid the morbidity associated with ALND.

Patients and methods: This prospective study was carried out on 50 females at the Breast Surgery Department in Ain Shams University Hospitals and El Helal Health Insurance Hospital after approval of the ethical committee.

Results: In our study, by combining the SLN technique with PLN technique using patent blue dye alone i.e. (without the radioisotope technique) we achieved high accuracy and less false negative rate by simple and cheap method. These techniques when done by an experienced surgeon can be used confidently in patients with early breast cancer.

Conclusion: We have found that combining PLN sampling with SLNB makes it possible to avoid ALND in a considerable proportion of breast cancer patients, thereby reducing postoperative morbidity, complications and the length of postoperative hospitalization.

Key words: Sentinel lymph node, axillary lymph node dissection, peripheral lymph node.

Introduction

Breast cancer still causes millions of women's lives to be disrupted. Breast cancer has been among the most common malignancies in women for a long time in terms of both incidence and death.[1]

According to recent data, there were 2.3 million new cases (11.7%) of breast cancer, with a 6.9% death rate. Incidence of breast cancer is higher in high-income nations (571/100000) than in low-income countries (95/100000).[2]

With 38.8% of all cancer cases in the population, breast cancer is the most frequent disease in Egypt among women. In 2020, there were over 22,700 instances of breast cancer, and by 2050, there were expected to reach 46,000 cases. According to estimates, 11% of people die from breast cancer.[3]

Numerous variables, including race, ethnicity, a positive family history, smoking, alcohol intake, physical inactivity, exogenous hormones, and specific female reproductive characteristics, increase the chance of developing breast cancer. Breast cancer risk is influenced by younger age at menarche, older age at first full-term pregnancy, and parity. Breast cancer has been linked to genetic mutations and variations.[4]

One of the most reliable indicators of a breast cancer patient's long-term prognosis is still the regional lymph node status. Although it might cause lymphedema, axillary lymph node dissection has historically been the most acceptable technique for determining metastatic spread to the locoregional lymph nodes. Since sentinel lymph node biopsy significantly lowers surgical morbidity without compromising diagnostic precision, it has taken the position of axillary lymph node dissection.[5]

Approximately 90% of axillary lymph node status may be predicted by sentinel node biopsy alone, according to several studies. Traditional axillary clearance has a significant risk of chronic symptoms, including lymphedema. It has been demonstrated that sentinel lymph node biopsy results in fewer issues. Several studies revealed reduced incidence of comorbidities in favor of SLN than ALND like arm morbidity, particularly lymphedema (5% vs. 13% and sensory loss (11% vs. 31%) and a better quality of life.[6]

False-negative results remain the primary issue. The false-negative rate in Sentinel lymph node biopsy trials published by the American Society of Clinical Oncology in 2014 ranged from 4.6% to 16.7%. According to a meta-analysis, the False Negative Rate of Sentinel Lymph Node sampling ranged from 0% to 29% on average. A task group was formed by the American Society of Breast Surgeons to provide appropriate guidelines for Sentinel Lymph Node Biopsy, they suggested in 2000 that the False

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Negative Rate should be kept to 5% or below and that the identification rate for Sentinel Lymph Node Biopsy be 85% or better. False-negative findings can have a variety of explanations, but one that is certain is that "skip metastasis" will lead to false negative results.[7]

This paradigm shift is associated with a controversial debate regarding the significance of axillary staging, the need for surgery, and the role of radiotherapy. Looking ahead, lymph node staging and axillary treatment might shift from sentinel lymph node biopsy with or without axillary dissection to ultrasound-guided needle biopsy and irradiation of regional lymph nodes to reduce complications such as lymphedema in early-stage breast cancer.[8]

So the management of axillary lymph node metastases is still under consideration in determining which solution is less mutilating, causing fewer complications, and with the best therapeutic rates.

Aim of work

We tried to assess the efficacy of combined SLNB using patent blue dye and PLN sampling in the management of nodal negative early breast cancer. We aimed to avoid the skip metastases phenomenon that occurs with sentinel lymph node biopsy and to avoid the morbidity and complications associated with axillary lymph node dissection using a simple and cheap method which can be available at any hospital.

Patients and methods

This prospective study was carried out on 50 females at the Breast Surgery Department in Ain Shams University Hospitals and El Helal Health Insurance Hospital after approval of the ethical committee.

Inclusion criteria: Females aged from 20 to 60 years. Females with nodal negative breast cancer both clinically and radiologically. Females with stage T1 and T2 Breast cancer. Females with no history of previous breast surgery. Females with a primary single breast mass.

Exclusion criteria: Females with a previous history of breast surgery. Females with multicentric and multifocal breast cancer. Females with metastatic breast cancer. Nodal positive breast cancer. Patients who received neoadjuvant chemotherapy. Patients diagnosed with secondary breast cancer, and those with concomitant other malignant tumors or significant organ lesions. Males with breast cancer.

Preoperative assessment:

All patients were subjected to the following:

Triple assessment:

Medical history and clinical examination: Detailed medical history is taken as (Age and sex, Habits, Gravidity, Parity, special habits of medical importance, body built, reproductive history and the onset of the disease. Family history of hereditary genetic mutations was also discussed. History of risk and predisposing factors like oral contraceptive pills, alcohol intake, etc.…). Physical Examination:

General medical examination: To detect any other metastatic lesions.

Detailed local examination: To detect breast masses and their relation to the surrounding structures, and axillary lymph nodes (which group, size, mobility, consistency and amalgamated or not).

Imaging: Sonomammography with a special comment on axillary lymph node status. MRI if indicated. Chest x-ray, Abd & Pelvis U/S.

Biopsy: Tru-cut biopsy for detecting the type of pathological findings. Hormonal receptors: Estrogen receptor (ER). Progesterone receptor (PR). Human epidermal growth factor receptor 2 (HER2). KI67 (Kiel 67).

Laboratory investigations: Complete Blood Count (CBC). Coagulation profile: PT, PC, INR. Electrolytes. Kidney function tests. Liver function tests. Random blood sugar. Virology.

Operative data: General anaesthesia was given. Patients are placed at the operating room table in the supine position, with the arm at a 90-degree angle abduction from the body. Sterilization of the surgical field. Injection of 2 cm3 patent blue dye **(Fig. 1)** subareolar **(Fig. 2)** and breast massage was done for 15 minutes.

Fig 1: A sample of Patent blue dye prior to injection.

Fig 2: Subareolar injection of PBD.

The patient's breast form, size, and lesion location were carefully considered while deciding whether to make a separate or same skin incision for the removal of the breast tumour. Breast lump removal and specimen labelling **(Fig. 3)** for examination by histopathologists (Short = superior, long = lateral, double = deep).

Fig 3: Excised Breast mass specimen after marking it for histopathologists.

A 5 cm lazy S incision was created at the lower border of the axillary hair region **(Fig. 4)**, and the skin flap was released toward the axilla. This incision was done on the same or different skin for axillary mapping.

Fig 4: Separate axillary incision.

Comprehensive examination of the axillary lymph nodes was conducted. The first blue-stained lymph node was classified as a sentinel lymph node **(Fig. 5)**. The node was excised and sent for histopathology.

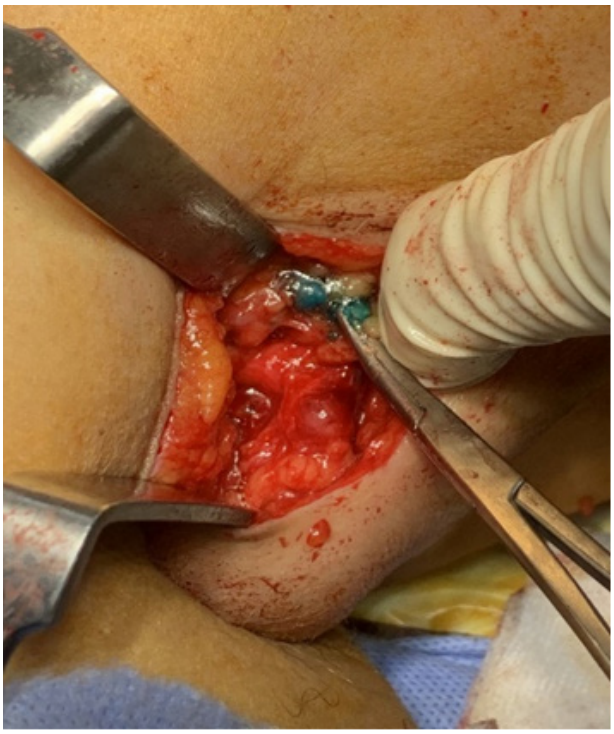

Fig 5: SLN: first encountered L.N. with blue dye.

The removal of suspicious lymph nodes in a field three centimetres surrounding the original lymph nodes was classified as PLN **(Fig. 6)**, which was sent apart from SLN.

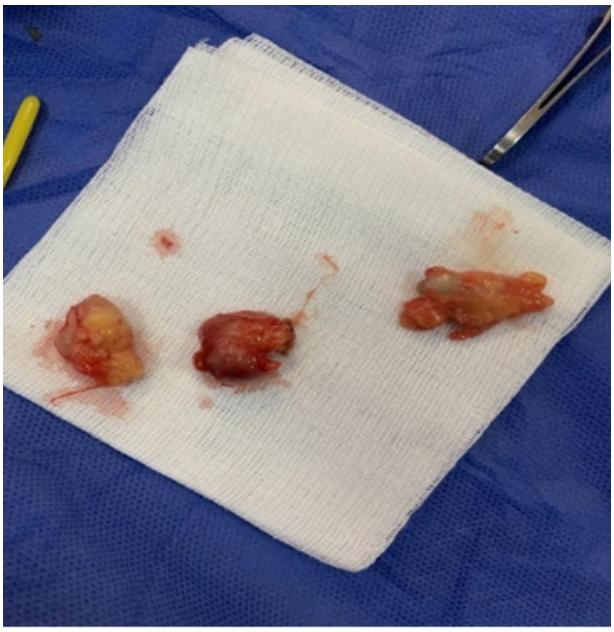

Fig 6: Peripheral L.N.s after excision.

The whole ALND (Level I plus level II lymph node dissection) was carried out according to the circumstances after the PLNs and SLNs were removed. All removed nodes were submitted for histological assessment following national guidelines. Nodes with isolated tumour cells (ITC) (≤ 0.2 mm), micrometastases (> 0.2 and ≤ 2.0 mm), or macrometastases (> 2.0 mm) were found to be either benign or involved. The placement of a suction drainage tube beneath the armpit following satisfactory hemostasis at the surgical site was decided according to the circumstances, and then closure of the axillary incision was done. Surgical bandaging followed surgery.

Observation indicators

Perioperative indicators: Perioperative data were carefully documented, including the length of the hospital stay, the surgery time, and the extubation time. Measurements were also made for postoperative drainage volume and intraoperative blood loss. Quality of life: During a 6-month follow-up period following surgery, the assessment took into account social, emotional, physiological, functional, and other aspects. Postoperative sequelae including oedema, numbness, hypoesthesia, subcutaneous effusion, and necrosis of the skin flap were also recorded.

Ethical considerations

The protocol was admitted to the ethics committee and administrator approval. Prior written consents were taken from the patients or their guardians. All data of patients were confidential with secret codes and private file for each patient. All given data were used for the current medical research only.

Statistical analysis

Statistical analysis was done by SPSS v26 (IBM Inc., Chicago, IL, USA). Shapiro-Wilks test and histograms were used to evaluate the normality of the data distribution. Quantitative parametric data were presented as mean and standard deviation (SD). Quantitative non-parametric data were presented as median and range. Qualitative variables were presented as frequency and percentage (%).

Results

The age ranged from 20 to 60 years with a mean value (± SD) of 41.44 (±10.37) years. 19 patients (38%) had average weight, 26 patients (52%) were obese and 5 patients (10%) were morbid obese. 8 patients (16%) had to drink coffee and 10 patients (20%) were smokers. The median of gravidity was 3 and ranged from 2 to 4. The median of parity was zero and ranged from zero to 1. Number of Lactating females was 12 patients (24%) and non-lactating females were 38 patients (76%).

20 patients (40%) had a family history of breast cancer. 15 patients (30%) weren't suffering from any disease, DM and HTN were present in 10 patients (20%), DM was present in 3 patients (6%) and HTN was present in 22 patients (44%).

The size ranged (L x W) from 0.5cm – 3cm x 0.5cm - 2.2cm with a mean (L x W) value (± SD) of 1.8cm ± 0.71cm x 1.5cm ± 0.52cm. Breast cancer was found at the lower outer quadrant in 1 (2%) patient, at the upper outer quadrant in 27 (54%) patients, at the lower inner quadrant in 10 (20%) patients, at the upper inner quadrant in 4 (8%) patients and at retro areolar in 8 (16%) patients. The onset of breast cancer ranged from 2 to 24 months with a mean value (± SD) of 7.8 (±6.81) months.

ER was positive in 37 (74%) patients. PR was positive in 32 (64%) patients. HER2 was positive in 8 (16%) patients. KI67 ranged from 5% to 67% with a mean value (± SD) of 24.5% (±17.02%).

For SLNB, the False negative rate = false negative / (true positive + false negative), so it = (b) / (a + b) = 2 / (15 + 2) = 11.76%. Sensitivity = true positive / (true positive + false negative), then it equals (a) / (a + b) = 15 / (15+2) = 88.2%. Accuracy rate = (true positive + true negative) / (true positive + false positive + true negative + false negative), so it = (a + d) / (a + c + d + b) = (15 + 33) / (15+2+0+33) = 96%. Negative predictive value = true negative / (true negative + false negative), then it = (d) / (d + b) = 33 / (33+2) = 94.2%.

For SLNB + PLN sampling, the FNR = (b) / (a + b) = 1 / (16 + 1) = 5.88%. Sensitivity = (a) / (a + b) = 16 / (16+1) = 94.11%. Accuracy rate = (a + d) / (a + c + d + b) = (16 + 33) / (16+1+0+33) = 98%. Negative predictive value = (d) / (b + d) = 33 / (1+33) = 97.05%.

Table 1: Demographic data of the studied patients

		n = (50)
Age (years)	Mean ± SD	41.44 ± 10.37
	Range	35 – 72
Weight	Average	19 (38%)
	Obese	26 (52%)
	Morbid obese	5 (10%)
Habits	None	32 (64%)
	Coffee	8 (16%)
	Smoker	10 (20%)
Gravidity	Median	3
	Range	2 – 4
Parity	Median	0
	Range	0 – 1
Lactation	Lactating	12 (24%)
	Non-Lactating	38 (76%)

Table 2: Family history and medical history of the studied patients

		(n=50)
Family history of Breast cancer	Negative	30 (60%)
	Positive	20 (40%)
Medical history	DM	3 (6%)
	HTN	22 (44%)
	DM+HTN	10 (20%)
	NonDM, Nor HTN	15 (30%)

Table 3: Size, site and onset of breast cancer of the studied patients

		No = (50)
Size	Mean ± SD	1.8cm ± 0.71cm X 1.5cm ± 0.52cm
	Range	0.5cm – 3cm X 0.5cm - 2.2cm
Site	Lower outer quadrant	1 (2%)
	Upper outer quadrant	27 (54%)
	Lower inner quadrant	10 (20%)
	Upper inner quadrant	4 (8%)
	Retro areolar	8 (16%)
Onset	Mean ± SD	7.8 ± 6.81
	Range	2 months – 24 months

Table 4: Hormonal tests of the studied patients

		(n=50)
ER	Positive	37 (74%)
	Negative	13 (26%)
PR	Positive	32 (64%)
	Negative	18 (36%)
HER2	Positive	8 (16%)
	Negative	42 (84%)
KI67 +/- (%)	Mean ± SD	24.5 ± 17.02
	Range	5 – 67

Table 5: Grouping based on the number of L.N.s

		1	2	3	≥4
-VE Mets	33	10	10	7	6
+VE Mets		2	2	1	1
SLN (+) / PLN (-)	6				
SLN (+) / PLN (+)	8	1	3	2	2
SLN (-) / PLN (+)	2	1	1	0	0
SLN (-) / PLN (-)	1	0	1	0	0
Total	50	14	17	10	9

Table 6: ALN metastasis state detected by SLNB

SLN Status	ALN Status		Total
	Positive	Negative	
Positive	15 (a)	0 (c)	15
Negative	2 (b)	33 (d)	35
Total	17	33	50

a = true positive, b = false negative, c = false positive, d = true negative.

Table 7: ALN metastasis state detected with SLNB and PLN sampling

SLN + PLN Status	ALN Status		Total
	Positive	Negative	
Positive	16 (a)	0 (c)	16
Negative	1(b)	33 (d)	34
Total	17	33	50

a= true positive, b= false negative, c= false positive, d= true negative.

Discussion

Sentinel lymph node dissection (SLND) alone is widely accepted as axillary management for women with clinically node-negative breast cancer. However, SLND without axillary lymph node dissection (ALND) for selected SLN-positive patients remains controversial.[9]

Sentinel lymph node dissection (SLND) makes axillary surgery more conservative and less morbid and improves the quality of life regarding; pain, lymphedema and shoulder stiffness.[9]

In this study, we conducted a validation study on the efficacy of combining Sentinel lymph node biopsy using the blue dye technique with Peripheral lymph node sampling in the management of nodal-negative breast cancer.

The main target of our study is to boost the accuracy of sentinel lymph node biopsy using a simple, available and cheap technique with less dependence on radioisotope technique which is not available across the whole country, enabling our surgeons against breast cancer.

In our study, we use patent blue dye which is a triarylmethane dye authorized as a food additive in the EU, that has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1970 and 1975 and the EU Scientific Committee for Food (SCF) in 1983.[10]

As regards the site for injection of the dye we prefer sub-areolar injection over peri-tumoral or any other method as it can be injected into the subareolar lymphatic plexus of Sappey and drainage is independent of tumour size and require less amount of the dye.[11-13]

This technique needs a strong learning curve and has been performed by relatively few surgeons with good experience in breast surgery.[14]

In this study, we included females with nodal negative breast cancer both clinically and radiologically, females with no history of previous breast surgery and females with primary single breast mass to avoid any method or route that can obscure or withhold the lymphatic pathway.

We excluded patients with a previous history of breast surgery, females with multicentric, multifocal

breast cancer and females with metastatic breast cancer, also females who received neoadjuvant chemotherapy because the axillary tissues feel different. Cancer cells are killed by chemotherapy and this produces an inflammatory response it is therefore not surprising that identification and dissection of sentinel nodes is a more difficult procedure after neoadjuvant chemotherapy and the sentinel lymph node biopsy identification rate is too low for routine use, and that the false-negative rate is also too high. We also excluded nodal-positive patients (Clinically and radiologically) because lymphatic pathways may be blocked and prohibit accurate mapping with PBD leading to a false negative result.[14-16]

The SLN identification rate during pregnancy and breast-feeding is just slightly inferior to the standard and the technique does not cause teratogenic effects. The onset of lactation must be pharmacologically blocked.[17]

In our study, we excluded males with breast cancer because of the rarity of cases and no sufficient previous studies about the drawbacks of SLNB in male breast cancer. However, some studies encourage SLNB in males with breast cancer.[18]

In our study, we found that among the total of 50 patients, the age group of BC-positive cases ranged from 20 to 60 years with a mean value (± SD) of 41.44 (±10.37) years. This was similar to the study done by Mahadevan et al., as they found that the age group of the total positive cases ranged from 20 to 85 years with an average age of 45.42±12.21. 19 patients (38%) had average weight, 26 patients (52%) were obese and 5 patients (10%) were morbid obese. These results match with many studies that reported that age and BMI are important factors in the non-visualization of SLNB. This is attributed to the replacement of breast tissue with fat which results in sluggish movement of dye in lymphatics and more attenuation in obese patients which obscure the visualization of SLN.[19-21]

We documented 8 patients had to drink coffee and 10 patients were smokers. Many studies observed no substantial association between coffee and the risk of breast cancer.[22,23]

We found that the number of Lactating females was 12 patients and nonlactating females were 38 patients. According to many studies; Breastfeeding reduces the risk of TNBC (20%) and in carriers of BRCA1 mutations (22–55%).[24]

We found that the upper outer quadrant was the most common site in 27 patients with a percentage of 54%, the lower outer quadrant masses were in 1 patient (2%), the upper inner quadrant masses were found in 4 patients (8%), the lower inner quadrant in 10 patients (20%) and retro areolar in 8 patients (16%). Wilting and Hagedorn reported in their study that left-oriented breast cancer (especially upper outer quadrant) showed 45.8% positivity.[25]

Estrogen receptor status showed that 37 patients were positive with a percentage of 74%, while 11 were negative (26%). Progesterone receptor status showed that 32 patients were positive with a percentage of 64%, while 18 patients were negative (36%). Her2/neu overexpression showed that 8 patients were positive with the percentage of 16%, while 42 patients were absent.

Additionally, the global percentage of Her-2 positive data ranged from 16 to 27%. Two populations, Germany and South Australia, were researched; the results showed that, respectively, 67.2 and 70.2% of the participants were positive for the ER receptor while 67.5 and 66.4% were positive for the PR receptor.[26-28]

Of the 50 patients in our research, 33 did not have ALN metastases and 17 did **(Table 5).** Out of the 17 patients, 6 had SLN positivity but no detectable peripheral nodes and 8 had SLN positivity with positive peripheral nodes. While only one patient was SLN negative and had no detectable positive nodes, the other two patients had positive peripheral nodes with negative SLN. The patients were categorized into four groups, based on the detection number of SLNs; N = 1, 2, 3, and ≥4 **(Table 5).**

Table 6 shows the results of SLNB for the 50 patients: sensitivity = 88.2% (15/17), accuracy = 96% (48/50), negative predictive value = 94.2% (33/35), and FNR = 11.76% (2/17). Table 7 shows the results after combining both techniques, the FNR reaches 5.88% (1/17), sensitivity = 94.11% (16/17), accuracy rate = 98% (49/50), and negative predictive value = 97.05% (33/34). After combining both techniques, there was a statistically significant change in FNR. Similar findings were obtained by Han et al. in their investigation; the FNR with SLNB was 9.9% and was considerably lowered to 4.2% upon combining PLNS with SLNB.[7]

35 of the patients underwent wide local excision (WLE), with a percentage of 70%, while 15 underwent a modified radical mastectomy with a percentage of 30%.

Drains after surgery were left 48 hours-72 hours for observation of reactionary haemorrhage. The mean amount of seroma on the first day was 250 cc, the second day was 150cc and third day was 50 cc.

Hospital stays in both procedures differed, in WLE the patients were discharged on the same day post-operative, while patients with MRM were discharged the day after surgery and were informed to be careful about avoidance of injection of the arm of the same side of the operation, movement of the shoulder

joint, care of drain and notice the colour and amount of discharge, We conclude that false-negative SLNB findings cannot be totally eradicated, taking into account both the state of SLNB technology today and the lymphatic metastatic pathway of breast cancer. According to published research, the incidence of "skip metastasis" in our study is "low level" within the usual range. An acceptable substitute for ALND is SLNB. The resultant FNR is still within a tolerable range even with "skip metastasis. There is another issue that should be taken into consideration for the cause of false negative SLN findings is the operator's learning curve. Therefore, in order to reduce the failure rate associated with the surgical experience of the operator, a multidisciplinary approach involving the surgeon, histopathologist, and nurse gaining the knowledge and skills necessary to enable successful technique and ensure the least FNR should be considered.

The current study was limited in a few ways. First, the purpose of the PLN sampling in a 3 cm diameter circle surrounding the first traced SLN was only a method to understand the lymphatic distribution in breast tissue. Until now there is no definite rule to account for the distance we estimated, and more research is needed to fully understand the anatomy of the lymph drainage pathway in this region. Second, a big research sample is required for additional examination because the sample size of a single group was small.

Since the turn of the twenty-first century, there have been significant changes in the treatment of breast cancer. The FNR of SLNB may be efficiently decreased by removing the PLNs that are within a specific radius of the SLNs. This can improve knowledge of SLNs and encourage the use of SLNB in conjunction with PLN sampling to lower the FNR and reduce the need for ALND in nodal negative BC. Nevertheless, we acknowledge that the FNR of SLNB will not drop to zero given the state of SLNB technology today and the "skip metastasis" phenomenon. Thus, using biological and pathologic tumour features in tandem to predict ALN status may become a new trend.

Conclusion

In our study, we try to use a simple, cheap and safe procedure by using patent blue dye for the staging of axillary lymph nodes and to avoid unnecessary lymph node dissection in nodal-negative breast cancer. We reached to reasonable result that can be safely applied to those patients with the least complications. In our study, we discussed one of the possible causes of failure of SLNB ` Skip metastases ` to be avoided in the future. We reported that training our surgeons is a very important issue to improve the result of SLNB in our institutes. Still, some questions should be answered in the future: is there any relation between coffee intake and the incidence of breast cancer, the relation between the distance where we should remove PLNs and less FNR, the lymphatic drainage of the area between breast tumour and sentinel lymph node and the phenomenon of "Skip metastases" needs to be further understood and evaluated. Finally, we recommend combining SLN with PLN sampling to achieve the best results with less morbidity and fewer complications for our women especially in rural areas who depend in their lives on manual work.

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