



Efficacy of the Management of Node Negative Early Stage Breast Cancer Using 1% Methylene Blue in Sentinel Lymph Node Biopsy: prospective study, Kasr Alaini University Hospital



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Abstract

Background: Accurate staging and proper management of axillary lymph nodes are important for the treatment of breast cancer. Sentinel lymph node biopsy (SLNB) provides accurate assessment of nodal status.

Aim of work: Evaluation the safety, the accuracy and the cost of the application of SLNB using methylene blue 1% (MB 1%) in cases of node negative early stage breast cancer. Patients and methods: This study was conducted at Kasr Al-Ainy university Hospitals, Faculty of Medicine, Cairo University. Fifty-two female patients with node negative early stage breast cancer who agreed on undergoing breast conservative surgery (BCS), SLNB using 1% methylene blue and frozen section examination then managed according to ACOSOG Z0011 criteria. Results: Identification in 50 surgical specimens (identification rate 96.15%). By evaluating frozen section examination of the lymph nodes in relation to paraffin section examination we found that the sensitivity of the frozen section examination was 100% and specificity was 97.5% with positive predictive value 90.9%, negative predictive value 100% and overall accuracy was 98%. To conclude: One % MB dye is very effective as a single agent in SLNB in node negative early stage breast cancer with very high identification rate compared to patent blue and radiocolloids. Implementation of ACOSOG Z0011 criteria proved its validity throughout our study as average 12 months follow up showed no locoregional recurrence.

Keywords: Sentinel lymph node biopsy; node negative; methylene blue; early stage breast cancer.

1. Introduction

Currently, Sentinel lymph node biopsy (SLNB) has become an accepted standard of care to stage the axilla for clinically node-negative early stage breast cancer¹.

Data from randomized trials have confirmed that patients treated with SLNB alone had significantly less arm and axillary pain, improved range of motion, less lymphedema, less numbness, fewer complications, and better quality of life than those treated with ALND².

Methylene blue is as good an SLNB agent as Isosulfan blue and is much cheaper. Addition of radio-colloid mapping to blue dye does not achieve a sufficiently higher identification rate to justify the

cost. Methylene blue is therefore the agent of choice for SLNB in developing countries³.

Methylene blue is also safe; with very rare reported cases of anaphylaxis. Local reactions in the breast characterized by erythema and necrosis have been reported with superficial injections of methylene blue. Methylene blue is an attractive alternative agent⁴.

The long-term follow-up data from the Z0011 study also revealed that the 10-year overall survival (OS) and disease-free survival (DFS) rates for patients who underwent SLND alone were not inferior to those for patients who underwent ALND. Furthermore, SLND alone provided excellent locoregional control based on early and late recurrence rates^{5&6}. Beside that omitting routine ALND after

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identification of a positive sentinel node resulted in residual axillary disease in 27% of patients, but the risk of axillary recurrence was less than 1%, thanks to that is the role of adjuvant radiotherapy ⁷.

2. Patients and methods

This prospective case series study was conducted at the Department of Surgery, Kasr Al-Ainy university Hospitals, Faculty of Medicine, Cairo University between December 1st 2017 and October 1st 2019. The study protocol was reviewed and permitted by the institutional research and ethics committee.

The study group included 52 female patients within the scope of our designed inclusion criteria and candidate for breast conservative surgery (BCS) female patients with early stage breast cancer with node negative axillary lymph nodes.

Fifty-two patients with clinically node negative early breast cancer who are candidates for Breast Conservative Surgery were studied. Inclusion criteria included Female patients with early stage breast cancer with node negative axillary lymph nodes, i: e (T1-2, N0, M0), while Exclusion criteria included Locally advanced cancer i: e (T3-4), Previous axillary surgery, Post neo-adjuvant chemotherapy, Patients with histopathology of ductal carcinoma in situ, Clinically positive axilla for palpable lymph node, clinically suspicious or pathologically proven by ultrasound and Contraindication of BCS as Multicentric breast cancer, Small breast and large affected area of pathological microcalcifications or architectural distortion and Patient's refusal.

Data were documented from medical records, including operative notes, radiology reports, and pathology reports.

Patients presenting to the breast clinic or referred from The National Screening Programme were subjected to triple assessment in the form of history taking, clinical examination, bilateral sonomammography and tru-cut needle biopsy of breast lesion. The patients were subjected to metastatic work-up (chest X-Ray and Pelvi-Abdominal Ultrasound) and Immunohistochemistry of the biopsy were done (ER and PR receptors, HER2neu, and Ki67). Negative axillary LNs (N0) were judged by the combination of clinical examination and US examination (typically showing LNs oval in shape with a smooth contour, a uniformly thin hypoechoic cortex 3mm or less, and an echogenic fatty hilum).

The patients' data was thereafter presented at the weekly multidisciplinary team (MDT) breast meeting where breast Oncosurgeons, Medical Oncologists, Radiologist and Pathology Consultants discuss the results of the investigations.

Patients who were categorized as node negative, early stage breast cancer (T1-2, N0) were involved in our study. Detailed informed consent was discussed with each patient regarding the discussion and decision that was taken through the MDT; informing her with PROs and CONs of the decision. Patients who accept to be enrolled in our study started the planned treatment within our premises.

Regarding the Technique of Sentinel Lymph Node Biopsy: Methylene blue dye 1% is used in SLNB, (100gm bottle powder cost 225-pound Algomhria company) (fig.1). The technique is simple; Intraoperative after induction of anesthesia, 5 ml of 1% dilute methylene blue is injected retroareolar(fig.2). After 15-20 minutes a small incision (2:3 cm) is placed in the axilla 1cm below the hair line.



Figure (1): Methylene blue powder used at our institute.



Figure (2):Retroareolar injection of MB1%.

To simplify identification of the SLNs, after incision of the axillary fascia the axilla is divided into four areas starting from the intersection of the second intercostobrachial nerve and lateral thoracic vein. Most of the SLNs are located inferior to the second intercostobrachial nerve and at the medial area of the lateral thoracic vein. SLN was successfully identified if a blue lymph node or a lymph node with a blue afferent lymphatic was visualized; then any enlarged axillary LN even if not stained blue is also removed. Finally closure, no drain.

The following were followed during the procedure: avoid intradermal injection (retroareolar was the method of our choice) and the surgical procedure of the axilla is to be performed within 15-20 minutes as delay in identifying SLN leads to false staining of next levels beyond true sentinel lymph nodes (Figs. 3&4)

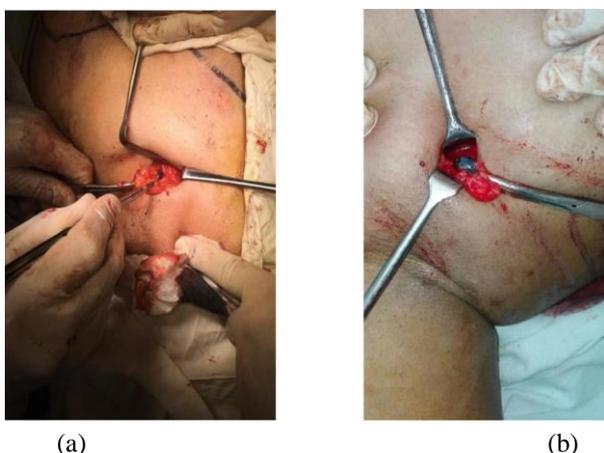


Figure (3) (a –b): Intra operative identification of the blue SLN 15-20 minutes after methylene blue injection (retroareolar) in two of our cases.

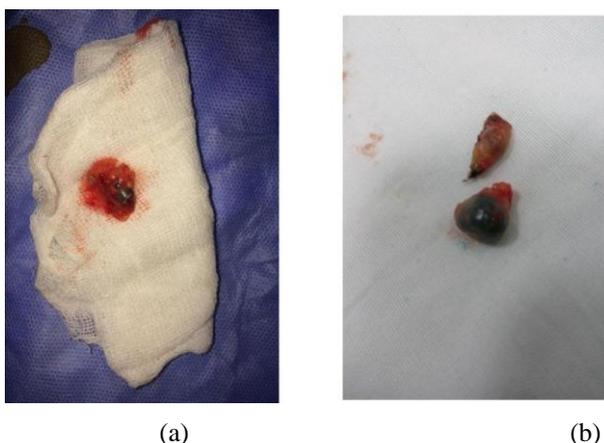


Figure (4) (a – b): Retrieved sentinel lymph nodes in 2 of our cases.

The lymph nodes are sent to the pathologist for frozen section. All SLNs were subjected to standard

FS evaluation with toluidine blue stained section. The SLN was bisected longitudinally and frozen. FS were taken with a microtome setting of 4 μ m. Macrometastases were defined as those having a diameter greater than 2 mm, micrometastases as those having a diameter between 0.2 and 2 mm, and isolated tumor cells (ITCs) as single tumor cells or small clusters of cells (diameter <0.2mm) for detection of positive or negative SLN to manage according to the ACOSOG Z0011 criteria where: negative SLNB: No further ALND, ITCs: No further ALND, Patients with micrometastasis or macrometastasis (1-2 out of 3-4 positive lymph nodes): No further ALND, Positive sentinel lymph nodes when the received nodes are less than three: ALND was performed and Patients with 3 or more retrieved lymph nodes if some of them are matted lymph nodes or lymph nodes with extra-capsular extension: ALND was performed.

The nodal tissue was fixed in 10% formalin and embedded in paraffin. After this fixation, serial sections were made of the SLN for definitive analysis with haematoxylin and eosin (H&E) and to confirm the final pathology results with Frozen section results. If more positive lymph nodes were discovered ALND is performed in another session. Patients were subjected to follow-up for detection of loco-regional recurrence and systemic metastatic rate and arm complications (lymphoedema, nerve injury and shoulder mobility restriction) for an average of 12 months. All patients had BCS with 1 cm safety margin, adjuvant RT and systemic therapy according to biological subtype.

3. Results

This study recruited 52 female patients with age ranged between 20 and 70 years; mean age 48.8 ± 11.9 years, with early stage breast cancer with node negative axillary lymph nodes, i.e (T1-2, N0, M0) who are candidates for Breast Conserving Surgery. Two cases showed failed sentinel lymph node identification, so excluded from our study.

Sentinel lymph node biopsy was done using 1% MB with lymph node identification in 50 surgical specimens (identification rate 96.15%). Two cases showed failed identification were excluded from the study. Number of lymph nodes identified in each specimen is shown as follows: six patients of the studied group (12%) have 6 lymph nodes, twelve patients (24%) have 5 lymph nodes, seven patients (14%) have 4 lymph nodes, twenty patients (40%) have 3 lymph nodes five patients (10%) have 2 lymph nodes.

Descriptive data:

The demographic and clinical variables in 50 women who were included in the study are shown in the following tables & figures:

Imaging data:

The average size of mass was (31.3 ± 11.4) mm; with (20%) in T1 stage, and (80%) in T2 stage.

The average cortical thickness of LN was (1.8 ± 0.6) mm; all LNs had preserved hilum.

Table (1): Imaging data among 50 women:

Variables		Mean ± SD
Size of mass (mm)		31.3 ± 11.4
Tumor size stage	T ₁	10 (20%)
	T ₂	40 (80%)
Cortical thickness of LN (mm)		1.8 ± 0.6
Hilum	Preserved	50 (100%)

Frozen section examination of the lymph nodes revealed 11 cases of the studied group (22%) with positive histological signs suggestive for malignancy, in these positive cases further surgical management was decided according to frozen section results as follows:

- Eight cases showed 1-2 positive lymph nodes out of 3-4 lymph nodes: No further ALND.
- Two cases showed 4 positive lymph nodes: ALND was performed.
- One case showed extracapsular extension: ALND was performed.

So, the number of positive cases (by frozen section examination) who required ALND was 3 cases out of 11 (27.2% of positive cases) and out of 50 (6% of total studied cases).

Paraffin section examination of the lymph nodes revealed 10 cases of the studied group (20%) with histological signs for malignancy. The 1 false positive case in frozen section examination did not need ALND, so the discordance between frozen section examination and paraffin section examination did not affect management of the cases.

Regarding malignancy detection; (22%) had positive frozen section results, (20%) had positive confirmatory paraffin section results, while none had recurrence after 12 months follow up.

Pathology data:

The average number of sentinel LNs was (3.8 ± 1.2).

Table (2): Pathology data among 50 women:

Variables		Mean ± SD
Number of sentinel LNs retrieved from all patients		3.8 ± 1.2
Frozen section results	+ve result	11 (22%)
Confirmatory paraffin section results	+ve result	10 (20%)
Recurrence rate	+ve result	0 (0%)

Diagnostic data:

Regarding diagnostic accuracy “based on confirmatory paraffin section”, frozen section had (98%) accuracy, (100%) sensitivity and (97.5%) specificity; with positive predictive value of (90.9%) and a negative predictive value of (100%).

Table (3): Diagnostic accuracy of frozen section vs confirmatory paraffin sections:

Variables	Frozen	Confirmatory paraffin
Diagnosis of malignancy (disease detection rate)	11 (22%)	10 (20%)
Accuracy (AUC) (%)	98%	100%
Sensitivity	100%	100%
Specificity	97.5%	100%
Positive predictive value (PPV)	90.9%	100%
Negative predictive value (NPV)	100%	100%

ROC (Receiver operating characteristic). AUC: Area under the ROC curve

Comparative studies:

The 50 women were classified according to malignancy detection in retrieved LNs “based on confirmatory paraffin section” into 2 independent groups:

- +ve malignancy group (10 patients)
- -ve malignancy group (40 patients)

Comparative study between the 2 groups revealed; highly significant increase in cortical LN thickness and size of mass, in +ve malignancy group; compared to -ve malignancy group; with highly significant statistical difference ($p < 0.01$ respectively).

Comparative study between the 2 groups revealed non-significant difference as regards age and number of sentinel LNs ($p > 0.05$).

Table (4): Comparison between the 2 groups as regards clinical and imaging data using Mann-Whitney's U and Chi square tests:

Variable	+ve group (10)	-ve group (40)	Mann-Whitney's U test
	Median (IQR)	Median (IQR)	P value
Age (years)	52.5 (48 – 64)	47.5 (40 – 59)	= 0.5121
Cortical thickness of LN (mm)	2.5 (2.1 – 2.7)	1.6 (1.2 – 2.1)	= 0.00061**
Size of mass (mm)	43.5 (30 – 48)	30 (21 – 37.5)	= 0.023*
Number of sentinel LNs	5 (3 – 5)	3 (3 – 5)	= 0.1488

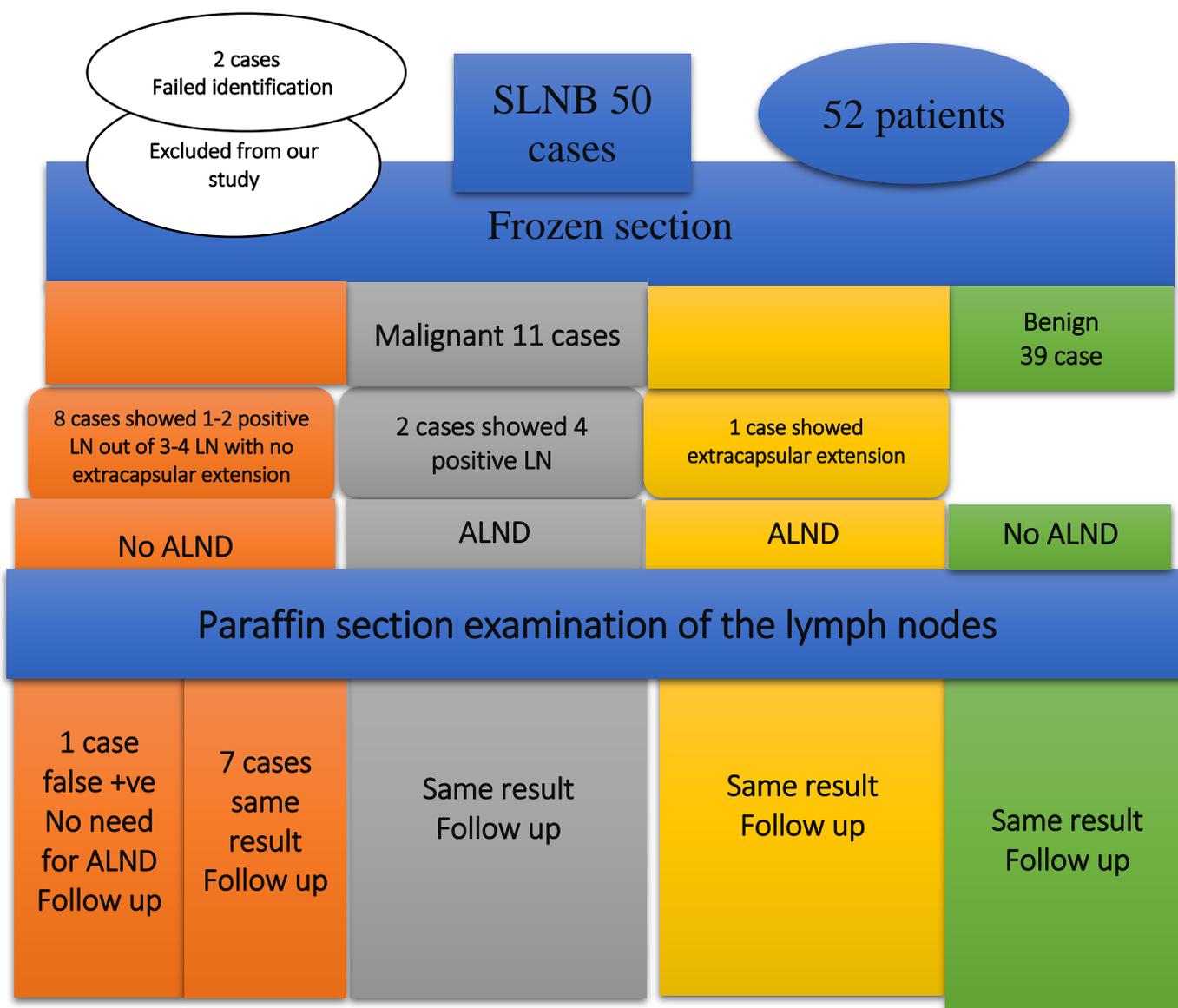


Figure (5): Illustration of the study.

IQR: inter-quartile range.

Paired comparative studies regarding (malignancy detection):

We analyzed and compared 50 (paired) group of patients according to the serial pathological assessments (frozen and confirmatory paraffin); data are shown in the following tables and figures:

➤ **Serial pathological assessments**

Comparative study between frozen and confirmatory paraffin assessments revealed; significant decrease in PPV in frozen section assessment, compared to confirmatory paraffin, with significant difference ($p = 0.029$).

Comparative study between frozen and confirmatory paraffin assessments revealed; non-significant difference in disease detection rate, accuracy, sensitivity, specificity and NPV ($p > 0.05$).

We found an excellent agreement between frozen and confirmatory paraffin assessments of malignancy detection ($\text{kappa} = 0.939$).

By using ROC-curve analysis, frozen section method predicted patients with malignancy, with excellent accuracy, compared to confirmatory. By using ROC-curve analysis, frozen section method predicted patients with malignancy, with excellent accuracy, compared to confirmatory paraffin method (98% vs 100%) ($p < 0.001$).

Table (5): Comparison between frozen and confirmatory paraffin as regards diagnostic accuracy assessments:

Variable	Frozen Assessment	Confirmatory paraffin assessment	McNemar's test
			P value
Diagnosis of malignancy (disease detection rate)	11 (22%)	10 (20%)	= 0.8070
Accuracy (AUC) (%)	98%	100%	= 0.3173
Sensitivity	100%	100%	= 1.000
Specificity	97.5%	100%	= 0.2629
Positive predictive value (PPV)	90.9%	100%	= 0.029*
Negative predictive value (NPV)	100%	100%	= 1.000

Table (6): An agreement between frozen and confirmatory paraffin assessments:

		Frozen		Total	Agreement	
		-ve	+ve		Kappa	p value
Confirmatory paraffin	Negative	39	1	40 (80%)	0.939	= 1.000
	Positive	0	10	10 (20%)		
	Total	39 (78%)	11 (22%)	50 (100%)		

Table (7): Roc-curve of each pathological method to predict patients with malignancy:

Variable	AUC	SE	Sensitivity (%)	Specificity (%)	P value
Frozen section results	0.988	0.0148	100	97.5	<0.0001**
Confirmatory paraffin section results	1.000	0	100	100	<0.0001**

ROC (Receiver operating characteristic), AUC= Area under curve, SE= Standard Error.

Known complications from 1%MB injection as superficial sloughing of the skin and permanent

pigmentation of the skin did not appear in any patient of the studied group.

Methylene blue was available in our institute each bottle 100gm powder which was enough for more than 100 patients cost 225 pounds, while patent blue is not widely available 2ml ampule cost 220

pounds for one patient 5ml ampule cost around 395 pounds needed for two patients.

Follow up of the patients for an average of 12 months showed no locoregional recurrence, lymphoedema, sensory loss nor shoulder movement restriction in any of the patients.

4. Discussion

Early stage breast cancer patients, with limited nodal involvement, should no longer be subjected to a completion ALND, based on the results of the IBCSG 23-01, AMAROS, and Z0011 trials⁸.

In this study Sentinel lymph node biopsy was done using 1% MB with lymph node identification in 50 surgical specimens (identification rate 96.15%). Two cases showed failed identification were excluded from the study. Management of SLNB positive cases was done following ACOSOG Z0011 criteria for management of SLNB in T₁-T₂, N₀ patients according to NCCN guidelines⁶.

Identification rates (IRs) for SLNB has increased over time from 88 % in 1992-2000 to 97% in 2007-2012. The increase in IR during the last 18 years is likely due to the increase in gained experience by the surgeons performing SLNB⁹.

This study used the procedure of retro-areolar injection and avoided intradermal injection. The site of administration of the dye plays a vital role in intraoperative identification and the retro-areolar route of administration of Methylene Blue is the best option for higher Identification Rates thereby increasing the efficacy of the Sentinel Lymph Node Biopsy technique^{10&11}.

This study, all lymph node specimens were reviewed by a dedicated breast pathologist by frozen section; it was found that frozen section had (98%) accuracy, (100%) sensitivity and (97.5%) specificity; with positive predictive value of (90.9%), a negative predictive value of (100%) and FNR (0%). Improved results which may be due to serial slicing of lymph nodes, examining multiple levels and proven expertise of the histopathologist, beside that in our study the mean tumor size was (31.3 ± 11.4) mm.

Although, the commonly used technique for intra-operative detection of sentinel lymph node metastases are frozen section (FS) examination, the sensitivity of FS examination of the sentinel lymph

node has been reported to range from 19.0% to 75%¹². False-negative SLN FS rates are variable, owing to variation in protocols and classification of isolated tumor cells, and are most often a result of sampling error and low-volume (isolated tumor cells or micro metastatic) metastatic disease¹³. It is believed that if all SLNs were bisected longitudinally for diagnosis separately it might be more effective in reducing the false-negative rate¹⁴.

In this study, injection of 1% MB was safe, no allergic or anaphylactic reactions were observed during the SLNB procedure, and most of the patients have a change in the color of urine to green blue for about 24 hours. This is also reported in most of the published studies, except Teknos et al., 2008, reporting a pulmonary oedema during a SLN procedure using MB¹⁵. The technique followed in this case series showed, no necrotic skin lesions, in contrast to published data, because of the retro areolar method of injection was the method of choice and not intradermal¹⁶. Some authors believe that complications seemed to be mainly due to intradermal injection of the blue dye more than related to dye concentration, intradermal injection leads to vasoconstriction which may end in skin sloughing due to inhibition of NO synthesis and prostacyclin release from the vascular endothelium¹⁷.

This study used Methylene blue, it is a cost-effective alternative to the Radioisotope technique with high accuracy, low false negativity and a high PPV. Moreover, methylene blue showed higher superiority regarding cost and good alternative to the gold standard patent blue. Although this forms a small fraction of the entire cost of SLNB, it does have a significant impact in the developing countries where there are budgetary funding constraints and patients are poor¹¹.

Isosulfan Blue and Patent Blue present with multiple side effects ranging from regional staining, rash at the site of administration to anaphylaxis and Methemoglobinemia. In this study no allergic reaction was observed using MB 1%. Therefore, among the available dyes, Methylene Blue is the safest¹¹. In contrast, Isosulfan Blue and patent blue have high protein binding capacity predisposing them to allergic reactions and possible life threatening anaphylaxis¹¹.

In 2019, Khallaf et al., studied 242 patients with (T₁₋₂, N₀, M₀) and SLNB using 1% MB was done with identification rate of 96.3%. 56 of 233

(24%) were positive for metastasis in LNs, Applying ACOSOG Z0011 guidelines only 12 required ALND (19.6%). No loco-regional recurrence was found after a follow up of average 18 months¹.

Guiliano et al., 2017, enrolled 891 women in randomized trial (446 in the SLND alone group and 445 in the ALND group). At a median follow-up of 9.3 years, between year 5 and year 10, one regional recurrence was seen in the SLND alone group vs. none in the ALND group. Ten-year regional recurrence did not differ significantly between the 2 groups implementing ACOSOG Z0011 Criteria¹⁸.

While meta-analyses documented a false-negative rate (FNR) of the SLNB of 5–7%, the incidence of regional (axillary) recurrence after a negative SLNB of 0.3–0.6%¹⁹.

Long-term follow-up results from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B32 study showed that the incidence of upper limb lymphedema in the ALND was 4 times that of the SLNB patients, significantly affecting the quality of life²⁰.

The number of positive cases detected by frozen section examination was 11 cases out of 50 (22.5%), of these 11 cases only 3 cases required ALND (27.2% of positive cases and 6% of overall cases). Applying the ACOSOG z0011 criteria to the patients in the case series, and follow up of them for an average of 12 months showed no loco regional recurrence nor metastatic occurrence in any of the patients in the study group. Moreover, follow up showed no nerve injury, restricted shoulder mobility nor lymphoedema in the study group.

This study has some limitations: as it described results of a single-center study with a small number of the studied population (a larger sample would better evaluate the efficacy and the accuracy of assessment of SLNB using 1% MB). Moreover, the Follow up of the patients for 12 months (follow up the study group for 5 or 10 years would have better impact on study results).

On the other hand this study has some strong points: as it described the SLNB early complication rate using MB1%, which is proved to be very safe drug without complications moreover, MB1% cost is proved to be minimal in comparison with other localization techniques with high identification rate.

5. Conclusion:

Using One % MB dye is very effective as a single agent in SLNB in node negative early stage breast cancer with very high identification rate compared to patent blue and radio colloids. Implementation of ACOSOG Z0011 criteria proved its validity throughout our study as average 12 months follow up showed no locoregional recurrence.

6. Declarations

Financial support and sponsorship: Nil.

Conflicts of interest: There are no conflicts of interest.

Ethical approval: The study was approved by the Institutional Ethics Committee

7. References

1. Khallaf E, Abdoon M, Lotfy A, Abdalaziz Y, Kamal R, Attia H. Methylene blue 1% as a sensitive and safe alternative for sentinel lymph node biopsy in early stage breast cancer: Results of a large pilot study. *Breast J.* 2019;(July 2018):1-3. doi:10.1111/tbj.13401
2. Gill G. Sentinel-lymph-node-based management or routine axillary clearance? One-year outcomes of sentinel node biopsy versus axillary clearance (SNAC): a randomized controlled surgical trial. *Ann Surg Oncol.* 2009;16(2):266-275.
3. East JM, Valentine CSP, Kanchev E, Blake GO. Sentinel lymph node biopsy for breast cancer using methylene blue dye manifests a short learning curve among experienced surgeons: a prospective tabular cumulative sum (CUSUM) analysis. *BMC Surg.* 2009;9(1):2.
4. Wu X, Lin Q, Chen G, et al. Sentinel lymph node detection using carbon nanoparticles in patients with early breast cancer. *PLoS One.* 2015;10(8):1-12. doi:10.1371/journal.pone.0135714
5. Ainsworth RK, Kollias J, Blanc A Le, Silva P De. The clinical impact of the American college of surgeons oncology group Z-0011 trial - Results from the BreastSurgANZ national breast

- cancer audit. *Breast*. 2013;22(5):733-735. doi:10.1016/j.breast.2012.11.005
6. Jung J, Han W, Lee ES, et al. Retrospectively validating the results of the ACOSOG Z0011 trial in a large Asian Z0011-eligible cohort. *Breast Cancer Res Treat*. 2019;175(1):203-215. doi:10.1007/s10549-019-05157-4
 7. Kang T, Yi M, Hunt KK, et al. Does blue dye contribute to success of sentinel node mapping for breast cancer? *Ann Surg Oncol*. 2010;17(3):280-285.
 8. den Hoven I, Klaveren D, Verheuel NC, et al. Predicting the extent of nodal involvement for node positive breast cancer patients: Development and validation of a novel tool. *J Surg Oncol*. 2019;120(4):578-586. doi:10.1002/jso.25644
 9. Niebling MG, Pleijhuis RG, Bastiaannet E, Brouwers AH, Van Dam GM, Hoekstra HJ. A systematic review and meta-analyses of sentinel lymph node identification in breast cancer and melanoma, a plea for tracer mapping. *Eur J Surg Oncol*. 2016;42(4):466-473. doi:10.1016/j.ejso.2015.12.007
 10. Dowlatshahi K, Fan M, Anderson JM, Bloom KJ. Occult metastases in sentinel nodes of 200 patients with operable breast cancer. *Ann Surg Oncol*. 2001;8(8):675-681.
 11. Rao JS, Ramesh R. Clinics in Oncology Peri-Areolar Administration of Methylene Blue Dye as an Independent , Cost Effective and Reliable Technique for Sentinel Lymph Node Biopsy in Early Breast Cancer. 2019;4:7-10.
 12. Lombardi A, Nigri G, Maggi S, et al. Role of frozen section in sentinel lymph node biopsy for breast cancer in the era of the ACOSOG Z0011 and IBCSG 23-10 trials. *Surgeon*. 2018;16(4):232-236. doi:10.1016/j.surge.2017.11.003
 13. Jorns JM, Kidwell KM. Sentinel lymph node frozen-section utilization declines after publication of American College of Surgeons Oncology Group Z0011 trial results with no change in subsequent surgery for axillary lymph node dissection. *Am J Clin Pathol*. 2016;146(1):57-66. doi:10.1093/AJCP/AQW078
 14. Qiao G, Cong Y, Zou H, et al. False-negative frozen section of sentinel lymph node biopsy in a Chinese population with breast cancer. *Anticancer Res*. 2016;36(3):1331-1338.
 15. Mathelin C, Croce S, Brasse D, et al. Methylene blue dye, an accurate dye for sentinel lymph node identification in early breast cancer. *Anticancer Res*. 2009;29(10):4119-4125.
 16. Stradling B, Aranha G, Gabram S. Adverse skin lesions after methylene blue injections for sentinel lymph node localization. *Am J Surg*. 2002;184(4):350-352.
 17. Salhab M, Mokbel K. Skin and fat necrosis of the breast following methylene blue dye injection for sentinel node biopsy in a patient with breast cancer. In: *International Seminars in Surgical Oncology*. Vol 2. Springer; 2005:26.
 18. Giuliano AE, Ballman K V., McCall L, et al. Effect of axillary dissection vs no axillary dissection on 10-year overall survival among women with invasive breast cancer and sentinel node metastasis: The ACOSOG Z0011 (Alliance) randomized clinical trial. *JAMA - J Am Med Assoc*. 2017;318(10):918-926. doi:10.1001/jama.2017.11470
 19. Roos MM, van Steenhoven JEC, Aalders KC, et al. Regional Recurrence Risk Following a Negative Sentinel Node Procedure Does Not Approximate the False-Negative Rate of the Sentinel Node Procedure in Breast Cancer Patients Not Receiving Radiotherapy or Systemic Treatment. *Ann Surg Oncol*. 2019;26(2):372-378. doi:10.1245/s10434-018-6940-5
 20. Zhou Y, Huang X, Mao F, et al. Predictors of nonsentinel lymph node metastasis in patients with breast cancer with metastasis in the sentinel node. *Medicine (Baltimore)*. 2019;98(1).