Original Article

Use of Methylene Blue Dye for Lymphatic Basin Mapping and Sentinel Lymph Node Biopsy in Breast Cancer Patients in Enugu, Nigeria

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Abstract

Background: Sentinel lymph node biopsy is the current standard of care for axillary staging and further treatment planning in patients with clinical axillary node-negative breast cancer. Sentinel node (SN) biopsy was designed to accurately stage the axilla and minimize the side effects of conventional axillary-lymph-node dissection without sacrificing oncologic outcomes. Sentinel lymph node biopsy is normally performed with nuclear scan and patent blue violet or isosulfan blue. These are expensive and not commonly available in resource-poor regions such as West Africa. Methylene blue dye is a commonly used agent in a wide range of clinical diagnostic procedures and has been used by other investigators to perform this procedure. This study was designed to demonstrate the feasibility and effectiveness of SN biopsy in the management of axillary node-negative breast cancer in resource-limited populations using methylene blue dye. **Aim:** To determine the efficacy of methylene blue dye as a single tracer in lymphatic basin mapping and sentinel lymph node biopsy in patients with clinical axillary node-negative breast cancer. **Methods:** This was a prospective, case-controlled study involving 28 consecutively presenting female patients with clinical axillary node-negative breast cancer at the University of Nigeria Teaching Hospital, Enugu. Each of the patients had lymphatic basin mapping and sentinel lymph node biopsy with a sub-areola-subdermal injection of methylene blue dye. The SN (s) were then removed using the dye as the marker. Each patient then had a mastectomy or wide local excision as appropriately planned and conventional levels I and II axillary dissection was performed in the same sitting. The SNs and other axillary nodes were reviewed independently by our institution's pathologist. Each patient's axillary dissection specimen acted as her control for the study. **Results:** The SNs were identified in 24 (85.7%) patients. There was a demonstrable learning curve with an improvement in identification rate in the later half of the cases (92.9%) compared to the earlier half of the cases (78.6%). A range of 1–3 nodes and a mean of 1.78 nodes were obtained. A sensitivity of 90.9%, specificity of 79.6%, false-positive rate of 28.6%, false-negative rate of 9.1%, and accuracy of 95.8% were obtained. There was no incidence of allergic/hypersensitivity reaction. **Conclusion:** Sentinel lymph node biopsy with methylene blue dye can be applied with high accuracy within resource-limited environments. However, there is a definite short learning curve that must be overcome and the procedure validated before clinical application in decision-making.

Keywords: *Axillary dissection, breast cancer, methylene blue dye, sentinel node*

Introduction

Breast cancer is the most common malignancy among Nigerian women, with an age-standardized incidence rate of 52 per 100,000 and 64.6 per 100,000 at Ibadan

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and Abuja cancer registries, respectively,[1] while Enugu cancer registry showed an age-standardized incidence rate of 60.3 per 100,000.[2] Clinical presentation of breast cancer in developing countries, such as Nigeria, is typically late with a relatively higher incidence in the younger age group (<50 years).[3] However, awareness is increasing and gradual stage migration is happening; hence, increasingly more patients present early.[3,4]

In the absence of distant metastases, axillary nodal involvement is the most important determinant of prognosis and ultimate survival. Axillary involvement is correlated with loco-regional recurrence, disease-free survival, and overall survival and guides further treatment options.[5-8] Obtaining information on the status of axillary involvement is an indispensable requirement for the proper management of any patient with early breast cancer.

Axillary-lymph-node dissection used to be the "gold standard" in the evaluation and management of axillary node involvement in patients with operable breast cancer.[9] However, it is associated with significant complications including Seroma collection, wound infection, injuries to nerves in the axilla, chronic shoulder pain, and most notably lymphedema.[10,11] Sentinel lymph node biopsy, a simple and minimally invasive staging procedure, has emerged as the standard of care in clinical axillary node-negative breast cancer patients.[5,12,13] Sentinel node (SN) biopsy was designed to accurately stage the axilla and minimize the side effects of conventional axillary-lymph-node dissection in patients with early breast cancer/clinical axillary node-negative disease. Sentinel lymph node biopsy takes advantage of the sequential metastases of breast cancer cells from levels I through II to level III axillary nodes. It has been found to better preserve the local anatomy with a positive impact on the quality of life without compromising oncologic outcomes such as overall survival and disease-free survival.[14,15] It enables sampling of the axillary basin with the removal of the first few lymph nodes draining the breast without removing all of levels I and II axillary lymph nodes.[16]

The modern lymphatic basin mapping technique involves the use of vital blue dyes, radiotracers, or a combination of both. The dual agent lymphatic basin mapping technique using the colorimetric method and radioisotopes have been universally accepted because of the higher SN identification rate and lower false-negative rates.[17,18] The use of the colorimetric method in areas without access to nuclear medicine facilities has also been validated.[13,19,20] Isosulfan blue and patent blue violet are the common agents for sentinel lymph node biopsy.[21,22] These agents are expensive, not readily available in the West African region and most centers lack the facility for the use of radiotracers. However, methylene blue dye is cheap and readily available, with fewer side effects when compared with other agents for sentinel lymph node biopsy. In this study, we aimed to ascertain the efficacy of methylene blue dye as a single-agent tracer in sentinel lymph node biopsy in patients with clinically axillary node-negative breast cancer.

Materials and Method

This study was conducted at the University of Nigeria Teaching Hospital (UNTH), Ituku-Ozalla, Enugu state. It was a prospective case-controlled study conducted over a 13-month period (October 2018–October 2019). We recruited 28 female patients with pathologically proven clinical T0 through T2, N0, and M0 invasive primary breast cancer, according to the American Joint Committee on cancer. All the patients underwent preoperative work up, consisting of a detailed clinical history and physical examination. Imaging studies such as breast and axillary ultrasound and mammography were performed. Core-needle biopsy was performed as a clinic procedure to obtain tissue for histologic diagnosis. The patients with suspicious axillary nodes underwent ultrasound-guided fine needle aspiration cytology of the lymph node to determine axillary nodal status. Other preoperative investigations that were performed include chest radiograph, computed tomography scan, serum electrolyte, blood urea and nitrogen, liver function test, complete blood count, echocardiography, and electrocardiogram. The axillary nodal status was confirmed negative prior to the commencement of neoadjuvant chemotherapy. Patients with clinical T3, T4, and N1 through N3 lesions, prior breast, and axillary oncologic surgery were excluded from this study.

Ethical approval for the study was obtained from the Health Research and Ethics Committee of the UNTH, Enugu, and written informed consent was obtained from each of the study participants.

One of the authors (OI) was involved as an assistant in 6 of the cases and the surgeon in 22 other cases. Adrenaline, hydrocortisone, and diphenhydramine were routinely available in an anesthetist's resuscitation kit in case of hypersensitivity reaction to methylene blue dye. The procedures were performed under general anesthesia with cuffed endo-tracheal intubation and multichannel monitoring, with the patient placed in a comfortable supine position and the arm abducted at 90 degrees on an arm board. A 3–5 mL of medical grade methylene blue was injected in the sub-areola-subdermal plane with a 25-G hypodermic needle, and a gentle massage

of the breast was performed for an average time of 5 min. This was followed by surgical preparation of the operating team, disinfection and draping of the operation site. The sentinel lymph node biopsy commenced about 15–30 min after this injection through a 4-cm transverse incision on the medial wall of the axilla, lateral to the pectoralis major. Careful sharp dissection was used to dissect through the axillary fat up until either a blue-stained lymphatic channel or first blue node (s) was identified. The dye-filled tract was then traced to the first echelon of blue lymph nodes. This node (s) was then excised as the SN. All blue nodes were taken as the SN (s). The lymph nodes so excised were then tagged appropriately with nonabsorbable sutures.

Afterward, completion of axillary dissection up to level II was done with mastectomy/lumpectomy in the same sitting. All the patients had levels I and II axillary dissection. The SN and other axillary nodes were sent separately to pathology and independently reviewed by the institution's pathologists, and the histology results of the SNs were compared with that of other axillary nodes.

The sensitivity, specificity, accuracy, false-negative rate, false-positive rate, negative, and positive predictive values of the SN procedure were calculated using the formulae described by Greenberg *et al*.[23]

Results

Twenty-eight patients with 28 tumor-bearing breasts satisfied the eligibility criteria for recruitment into this study. Their ages ranged from 26 to 74 years (mean: 46.6). Seventeen (60.7%) of these women were premenopausal, while 11 (39.3%) postmenopausal. Mastectomy was done for 23 (82.1%) of these patients, while 5 (17.9%) had breast-conserving surgery [Table 1].

The mean breast tumor size was 2.5 ± 1.95 cm with a range of 0–5 cm [Table 2]. Seventeen of the tumors were in the left breast (60.7%) and 11 were in the right breast (39.3%). These tumors were localized in the upper outer quadrant (35.4%), central (21.4%), upper inner quadrant (17.9%), lower inner quadrant (14.3%), and lower outer quadrant (10.7%). The predominant histologic subtype is invasive ductal carcinoma (85.7%), invasive lobular carcinoma (7.1%), and 3.6% each for medullary and mucinous carcinomas. The immuno-histochemistry result was available in 26 (92.9%) of our patients. In total, seven (25%) of the patients had hormone receptor (ER^+ PR^+) positive human epidermal growth factor receptor 2 negative disease.

The overall SN identification rate was 85.7%. The identification rate in the first half of the cases was 78.6 and 92.9% in the second half [Table 3]. The sentinel lymph node could not be identified in four patients. A mean of 1.78 ± 0.619 nodes and a range of 1–3 nodes were obtained during the SN biopsy, while the mean number of axillary nodes removed during the completion of axillary dissection was 11.25 ± 3.49.

Table 1: Patient characteristics

Characteristics	Mean(SD)	No(*n*=28)	%
Age (years)	46.6(11.49)		
Menopausal status			
Premenopausal		17	60.7
Postmenopausal		11	39.3
Type of biopsy			
Core-needle		18	64.3
Excision		10	35.7
Type of surgery			
Mastectomy		23	82.1
Breast-conserving surgery		5	17.9

Table 2: Tumor characteristics

Characteristics	Mean(SD)	No (*n*-28)	%
Tumor size	2.45(1.95)		
Tumor location			
Left breast		17	60.7
Right breast		11	39.3
Upper outer quadrant		10	35.7
Upper inner quadrant		5	17.9
Lower inner quadrant		4	14.3
Lower outer quadrant		3	10.7
Central		6	21.4
Tumor histology			
Invasive ductal carcinoma		24	85.7
Invasive lobular carcinoma		2	7.1
Mucinous		1	3.6%
Medullary		1	3.6%
IHC Subtype			
ER+/PR+/HER2−		10	35.7
ER+/PR+/HER2+		4	14.2
ER−/PR−/HER2+		5	17.9
ER−/PR−/HER2−		7	25
NO IHC		2	7.1

IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma; IHC: Immunohistochemistry ER: estrogen receptor; PR: progesterone receptor; HER2: Human Epidermal Growth factor receptor

Metastases were detected in both the SNs and axillary nodes in 10 patients. Four patients had metastases in the SNs but not in other axillary nodes. One patient had metastases in only the axillary node and none in the SN (s). Nine patients had no metastasis in both axillary node and the SNs [Table 4].

The SN identification rate was 85.7%, with a sensitivity of 90.9, the specificity was 69.2%, false-negative rate

Table 3: Intraoperative findings

	Mean (range)	No	%
Successful SN identification			
First half (*n*=14)		11	78.6
Second half (*n*=14)		13	92.9
Total (*n*=28)		24	85.7
Mean no of nodes in SN	1.78±0.62 (1-3)		
Mean no of nodes in ALND	11.25±3.49 (5-17)		
Surgical procedure			
Mastectomy		23	82.1
BCS		5	17.9

SN, Sentinel node; AN, Axillary node; BCS, Breast-conserving surgery

Table 4: Comparison of histology results of sentinel lymph node (s) and other axillary nodes

		Yes	No	Total
Sentinel	Yes	10	4	14
Lymph node	No	1	9	10
Metastases	Total	11	13	24

ALND, axillary lymph nodal dissection

Table 5: Sensitivity, specificity, false-negative and false-positive rates, accuracy, negative predictive value, and positive predictive value

	n	%
Identification rate	24/28	85.7
Sensitivity	10/11	90.9
Specificity	9/13	69.2
False positive	4/13	30.7
False negative	1/11	9.1
Accuracy	23/24	95.8
Negative predictive value	9/10	90
Positive predictive value	10/14	71.4

Table 6: Complications of sentinel lymph node biopsy using methylene blue dye

Complication	No (*n*=28)	%
Urticaria	0	0
Anaphylaxis	0	0
Transient blue skin tattooing	1	3.6
Flap necrosis	1	3.6
Surgical site infection	1	3.6
Blue urine coloration	28	100

was 30.75, and while the false-positive rate was 9.1%. The accuracy rate was 95.8%. The negative predictive value was 90%, while the positive predictive value was 71.4% [Table 5].

Table 6 shows the list of postoperative complications encountered during the course of this study. No allergic/hypersensitivity reaction was noted. One patient that had breast-conserving surgery had bluish skin discoloration, which lasted about 1 week. Flap necrosis and surgical site infection each were noted in one (3.6%) of the patient. All the patients had blue urine discoloration, which cleared within 24–48 h.

Figure 1: Intraoperative picture of the procedure

Discussion

This study evaluated the utility of single-agent SN biopsy in a standard cohort of breast cancer patients in Nigeria who presented with early disease. The mean age of the study participants was 46.6 ± 11.49 years. The majority of the patients, 17 (60.7%), were premenopausal at the time of diagnosis and treatment. This relatively younger age at presentation has been demonstrated by several authors within Nigeria.[3,4,24] Ezeome noted a marginally lower mean age at presentation of 45.7 years within the same region.[4]

Invasive ductal carcinoma was the commonest histologic variety and accounted for 85.7% of cases. Titiloye *et al*.,[25] in their assessment of the histology of breast cancer subtypes in Nigeria, noted that invasive ductal carcinoma was the predominant subtype and accounted for over 80% of cases. The mean pathologic tumor size obtained in this study was 2.45 ± 1.95 cm and 17% had breast-conserving surgery. However, in a work by Ogundiran *et al*.[24] at University College Hospital, Ibadan, they noted that only 2.1% of the patients had breast-conserving surgery between 1999 and 2009. The low volume of the tumor is not only in keeping with the early presentation but also demonstrates the added value of neoadjuvant systemic treatment as used in the management of majority of the patients in this cohort. The rising demand for breast-conserving procedures observed may be due to the increasing awareness with consequent earlier diagnosis and treatment.

The cost effectiveness and availability of methylene blue dye in the local Nigerian market influenced our decision to use this agent for this study. Studies have demonstrated the equivalence of methylene blue dye to the commonly used alternatives (isosulphane blue and patent blue V) in terms of outcomes such as SN identification rate and the number of identified SNs.[13,20,26] Another putative advantage of methylene blue dye is the significantly lower risk of life-threatening adverse reactions.[13,22,26,27] The unavailability of nuclear medicine facility in our center strengthened our quest to evaluate the outcome of a single tracer methylene blue dye in SN biopsy in our hands. Kavallaris *et al*.[19] had noted that methylene blue dye is a reliable single agent for sentinel lymph node mapping in areas without access to nuclear medicine facilities.

A sub-areola-subdermal injection technique was used irrespective of the tumor location in the breast. This enabled intraoperative visualization of the lymphatics and facilitated the SN identification, as shown in Figure 1. Intradermal injection techniques have been shown to be equivalent to the peritumoral injection technique.[28,29] Klimberg *et al*.[29] noted a 100% concordance rate following combined peritumoral radio-colloid and intradermal dye injection techniques. This injection technique is advantageous in patients with clinically impalpable breast tumor, which may make peritumoral injection difficult except with intraoperative imaging techniques such as ultrasonography, an imaging modality that is not readily available in our operating suites.

A mean of 1.78 ± 0.62 nodes and a range of 1–3 nodes were harvested in this study. This is similar to the findings in several other studies where methylene blue was used as a single-agent tracer in lymphatic basin mapping and SN biopsy.[13,19,20,26,29,30] The initial consideration that the relatively lower molecular weight of methylene blue dye (319.9) versus isosulfan blue could lead to the diffusion of methylene blue dye beyond the SNs, with consequent retrieval of more nodes was found not to be so. Varghese *et al*.[13] similarly noted that there was no significant difference in both identification rate and the number of SNs harvested when methylene blue was compared with isosulfan blue.

The American Society of Breast surgeons recommends a sentinel lymph node identification rate of 95% and a false-negative rate of 5–10%.[31] However, we obtained a SN identification rate of 85.7% and a false-negative rate of 9.1%. The failure to identify the SN (s) assigns such patient to conventional axillary dissection with its associated complications. There was an improvement in the SN identification rates between the first half of cases (78.6%) and the second half of cases (92.9%). This demonstrates a learning curve, as noted by Somasundaram *et al*.[32] All recruited participants had levels I and II axillary dissection as control. The SNs and axillary nodes were then sent independently for pathologic evaluation. This was done to give the patients the best quality care and, at the same time, enable the investigators to validate the procedure, overcome the learning curve, and be adept at the procedure before clinical decision-making will be based on the procedure.

In this study, the SN was the only positive node in four patients who were found to have no nodal metastasis on axillary node dissection (false-positive rate = 28.6%). This is not a true false-positive rate but rather represents the group of patients with axillary metastasis only in the SNs without other axillary nodes infiltrated. Several studies have demonstrated that the false-positive rate value may be as high as 60%.[33] These patients will derive no benefit from further axillary dissection, but there is no way of demonstrating the absence of disease in the rest of the axillary nodes in these patients except with conventional axillary dissection.

Nine patients were found on both SN and axillary dissection to harbor no axillary disease. These were the true-negative cases that would not benefit from axillary dissection. This group would have been spared unnecessary further axillary dissection and its associated co-morbidities if treatment decisions were made with the result of this SN dissection.

A false-negative rate of 9.1% and an accuracy of 95.8% were noted in this study. The false-negative rate was within the recommended level of 5–10% by the American Society of Clinical Oncology.[31] The potential risk of a false-negative SN biopsy is understaging and an increased risk of long-term axillary recurrence. False-negative rates have been noted to be higher in patients who received preoperative systemic treatment, especially in clinically node-positive disease patients.[33,34] This is thought to be as a result of heterogeneous response in patients with an axillary disease, which may cause rerouting of lymphatics with consequent lower identification rate and high false-negative rate. Skip metastases (nonsentinel axillary-lymph-node metastases without SN involvement) are an unavoidable cause of false-negative rate. However, this has been noted to occur in less than 0.1% of cases.[35] The false-negative rate obtained from this study is within the recommended range, notwithstanding that 60.7% of our patients had neoadjuvant treatment. It must be noted, however, that those selected for this study had clinically node-negative disease prior to commencement of neoadjuvant treatment. Hunt *et al*.[36] in a multicenter trial had noted no statistical difference in SN identification

rate and false-negative rate in patients with clinically node-negative T1–T3 breast cancers before and after neoadjuvant chemotherapy. They concluded that sentinel lymph node biopsy after chemotherapy decreases the number of positive SNs and unnecessary axillary dissection.

Allergic and possibly life-threatening anaphylactic reactions are the most dreaded complications of blue dyes used in lymphatic basin mapping. This has been noted to occur in about 1–3% of patients when isosulfan blue or patent blue V dyes were used in lymphatic mapping.[13,22,26,27] There was no recorded incidence of adverse hypersensitivity reaction to methylene blue dye in this study. Several studies have buttressed the better safety profile of methylene blue when compared to other dyes used in lymphatic basin mapping.[13,18,27,37] Following parenteral injection, methylene blue dye is rapidly absorbed in plasma and excreted in urine.[38] This was noted as bluish discoloration of urine, which occurred in all our patients and lasted for about 24 hours and abating within 48 hours. Skin-related complications such as skin necrosis is common with methylene blue dye compared with other colorimetric agents used in SN biopsy.[37] Stradling *et al*.[39] noted skin necrosis in 21% (5 of 24) of cases following intradermal injection of methylene blue dye. In this study, only a single patient out of the 28 subjects had flap necrosis following mastectomy. The incorporation of the nipple–areola complex in the horizontal elliptical mastectomy incision after subareola-subdermal injection of methylene blue dye and lymphatic mapping may partly explain the low incidence of skin-related complications in this study. Also, in the five patients that had breast-conserving surgery, there was no incidence of skin necrosis, but there was a single case of bluish skin tattooing which disappeared after about 1 week.

With the ever-expanding population of patients with early breast cancer and better awareness of the complication of axillary clearance, it has become important to establish lymphatic basin mapping and sentinel lymph node biopsy as the standard in the management of our patients who present early in resource-limited environments. This study has established the feasibility of this procedure and the rapid gain in proficiency as a means of axillary staging, and to determine the need for further loco-regional and systemic treatment for patients who present early with clinically negative node axillary node breast cancer in resource-limited environments.

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Conflicts of interest

There are no conflicts of interest.

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