



## Peri-Areolar Administration of Methylene Blue Dye as an Independent, Cost Effective and Reliable Technique for Sentinel Lymph Node Biopsy in Early Breast Cancer

Joseph Sushil Rao and Rakesh Ramesh\*

Department of Surgical Oncology, St. John's Medical College Hospital, India

### Abstract

**Background:** Sentinel Lymph Node (SLN) biopsy is the standard technique of pathologically staging a clinically negative axilla for decisions on adjuvant treatment. Multiple techniques and materials can be used to identify sentinel lymph nodes, of which, Blue Dye or Radio isotopes are commonly used. Due to high costs of radioisotope techniques, dyes are preferred in the underdeveloped countries. Studies demonstrate dye techniques being equally effective when compared to radioisotopes. In our study, we analyze the efficacy of Methylene Blue for identifying the SLN in clinically negative axilla of early breast cancer patients and determine the best site of administration for superior identification rates.

**Methodology:** The prospective study between May 2015 to April 2016 comprised of 40 newly diagnosed early breast cancer patients undergoing treatment in the Department of Surgical Oncology of a tertiary medical center in South India. Following Institutional Ethical Committee clearance and informed consent, 3 ml of 1% Methylene Blue was injected in the peri-areolar/peri-tumor area of the affected breast. The SLN was identified intra-operatively, and the data was statistically analyzed using SPSS version 24.

**Results:** Accuracy of identifying the SLN was 94.7% with a false negative rate of 5.26%. The technique demonstrated a sensitivity and specificity of 89% and 100% respectively with a positive predictive value of 100% and negative predictive value of 93.3%. The Identification Rate of Peri-areolar administration (96%) was significantly (0.003) superior to the Peri-tumoralsite (79%).

**Conclusion:** The high accuracy rate with low false negative rate of Methylene Blue suggests a reliable alternative to radioisotopes or a combination of Radioisotopes and Dye techniques for the detection of SLN biopsies in early breast cancer patients with clinically negative axilla. The Peri-areolar route of administration should be used to attain better results and higher efficacy rates.

**Keywords:** Early Breast Cancer; Sentinel Lymph Node; Methylene Blue Dye

### Introduction

The post-operative period following Axillary Lymph Node Dissection (ALND) is associated with high morbidity [1] thereby resulting in the possible increased duration of hospitalization. Sentinel Lymph Node (SLN) biopsy was a revolutionary technique introduced in the early 1990s [2], which over the years has been accepted as the standard of care in early stages of breast cancer [3], lowering the morbidity and duration of hospital stay.

The first lymph node receiving lymphatic drainage from the malignancy is termed as the Sentinel Lymph Node. Other lymph nodes can also receive tumor metastasis thereafter. Therefore, if there is no lymphatic metastasis in the SLN, it is confirmed that there are no other lymph nodes that can have metastasis [4].

In 1994, 174 patients diagnosed with breast cancer were involved in a study, of which SLN was identified in 114 (65.5%). The metastasis to the SLN was confirmed in 109 of the 114 (95.6%) patients. The False Negative Rate (FNR) decreased to 0% in their subsequent studies [5].

Isosulfan Blue, Patent Blue and Methylene Blue are few of the dyes that are routinely used for SLN biopsy [6]. Isosulfan Blue and Patent Blue present with multiple side effects ranging from regional staining, rash at the site of administration to anaphylaxis and Methemoglobinemia [7-9]. It has recently been established that Isosulfan Blue acts as an antigen and promotes IgE mediated Type

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#### \*Correspondence:

Rakesh Ramesh, Department of Surgical Oncology, St. John's Medical College Hospital, Bangalore, 560034, India, Tel: +91-9980007455; E-mail: srakesh99@yahoo.co.in

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I Hypersensitivity reactions [10]. Therefore, among the available dyes, Methylene Blue is the safest [11]. Methylene Blue is also used as an antidote in severe methemoglobinemia by the mechanism of methemoglobin reductase system [12]. The cost of Methylene Blue represents about 3% of the cost of the other two dyes and is also readily available in majority of the hospitals in the developing countries; making it the most commonly used dye for SLN biopsy [13].

The route of administration of the dye is also controversial and we believe there are no guidelines for the desired route while using Methylene Blue. Studies using Indocyanine Green fluorescence [14], Radioisotopes [15] have studied the lymphatic mapping during SLN biopsy. However, Methylene Blue has different structural properties [16] when compared to the other dyes and there is a need to define the best route while using Methylene Blue.

In our study we analyze the efficacy of using Methylene Blue for identifying the SLN in clinically negative axilla of early breast cancer patients and compare the routes of administration of dye to improve the sensitivity and specificity of the technique.

## Materials and Methods

In this prospective study between May 2015 to April 2016, 40 women recently diagnosed of early breast cancer with a clinically negative axilla in the Department of Surgical Oncology at a tertiary medical center in South India were included in the study after obtaining Institutional Ethical Clearance and preoperative consent. As part of the triple assessment in the diagnosis of breast cancer, mammography and core biopsy to estimate the receptor status (Estrogen Receptor/Progesterone Receptor and Her2-neu) were performed for all the patients. Those presenting with a clinically palpable metastatic lymph node or patients who underwent prior axillary surgeries were excluded from the study. Follow up of all patients was done by the principal investigator who was present in the operation theater.

All patients were administered 3 ml of 1% Methylene Blue in the peri-tumoral/peri-areolar region and the breast was massaged for five minutes to facilitate lymphatic spread of the dye to the draining lymph nodes. Following the administration of Methylene Blue and obtaining frozen section diagnosis on the excised SLN, either a Modified Radical Mastectomy (MRM) or a Breast Conservative Surgery (BCS) was performed as scheduled to further determine the histopathological diagnosis of lymphatic metastasis. After injecting the dye, the SLN was first identified and excised before the tumor was surgically addressed. The SLN was identified by visualization of the blue pigment on the lymph node. The SLN was excised during axillary surgery and sent to the Department of Pathology in normal saline for a frozen section to determine the pathological staging to either proceed with the clearance of lymph nodes when positive or to abandon axillary clearance when the SLN was negative for metastasis. The tumor specimens along with the dissected axillary lymph nodes were histopathologically evaluated postoperatively in the Department of Pathology and metastasis rates were statistically analyzed using SPSS version 24.

## Results

The mean age of the 40 patients involved in this study was  $49.7 \pm 12.718$ . Modified Radical Mastectomy was performed on 24 patients (60%) and Breast Conservative Surgery on 16 patients (40%). Table 1 describes the tumor locations. Four (10%) patients presented with Paget's disease of the breast. The largest tumor was 5 cm  $\times$  4 cm in

**Table 1:** Tumor Location.

Left Breast	24 (60%)
Right Breast	16 (40%)
Supero-lateral	26 (65%)
Supero-medial	4 (10%)
Infero-lateral	4 (10%)
Central	6 (15%)

**Table 2:** Statistical Analysis of SLN Identification.

Accuracy	94.70%
Sensitivity	89%
Specificity	100%
False Negativity	5.26%
Positive Predictive Value (PPV)	100%
Negative Predictive Value (NPV)	93.30%

**Table 3:** Comparison of SLN Positivity with Histopathological diagnosis between the two different routes of dye administration.

Route of Administration	SLN Positivity	Histopathology
Peri-areolar	4/20	4/20
Peri-tumoral	4/20	6/20

dimension and the smallest tumor was 1.5 cm  $\times$  1 cm.

In eight (20%) patients, only one SLN was identified. No metastasis was found in either the SLN or the axilla in 28 (70%) when a minimum of one SLN was identified. The SLN positive for metastasis demonstrated uptake of dye in maximum of three and a minimum of two lymph nodes. The rates of accuracy, sensitivity, specificity, False Negativity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) are described in Table 2.

The site of administration of the Methylene Blue dye was equally distributed among the total number of patients (Peri-tumoral and Peri-areolar with 20 each). The mean number of lymph nodes identified using the Peri-areolar route was 1.9 (SD=0.553; SE=0.124) with the maximum number of lymph nodes identified being three and minimum of one. With respect to the Peri-tumoral route, the mean number of identified lymph nodes was 1.9 (SD=1.483; SE=0.332) with the maximum number of lymph nodes identified in one patient being five. Four patients had no uptake of the dye. The Identification Rate (IR) for Peri-areolar injection of 96% was significantly ( $p=0.003$ ) higher than the Peri-tumoral injection group with an IR of 79%.

Identification of lymphatics was compared between the two routes of administrations. The Peri-areolar route identified the lymphatics in all of the patients while the peri-tumoral route failed to identify in six patients. Table 3 compares the SLN success rate with the histopathological report of the node clearance.

Four nodes that showed uptake failed to demonstrate histology and a repeat nodal frozen section biopsy was done to determine metastasis.

According to the TNM (Tumor, Node, Metastasis) Classification, both the false-negative SLN patients in the Peri-tumoral group were T3 N1 M0.

## Discussion

Post-operative lymphedema, pain, restriction of movement, and

possible loss of sensation can often result in morbidity and thereby resulting in increased duration of hospitalization. Intra-operative complication such as vascular injury and damage to the nerves can often lead to decrease in the quality of life in the post-operative period [17]. SLN biopsy offers less complication by decreasing the extensive dissection in contrast to ALND. Metastasis to the axillary lymph nodes is not detected in 95% to 97% of the T1 tumors and 52% to 77% of T2 tumors [18].

SLN biopsy can be performed either by using a radioactive material or by using dyes based on visual staining. In the former, the radioactive material is injected into the breast 12 hrs prior to surgery and a gamma probe is used intra-operatively to identify the SLN. When a dye is used, the administration of the dye is done at the start of the procedure and the SLN is detected based on the uptake of the colored dye intra-operatively. However, the two techniques can be used together to obtain the best results with the highest accuracy [13,17].

Radioactive technique for the identification of the SLN was introduced in the year 1993 [19] and since then the technique has been utilized all over the world for SLN biopsy. However, radioactive material is expensive and most of the patients and hospitals in resource poor settings would not be able to afford them. However, Methylene Blue is cheap and can be safely [10] used in place of Isosulfan Blue or Radio-isotopes providing good efficacy in SLN identification [20-22]. With a specificity and PPV of 100%, our study confirms the efficacy of Methylene Blue as a simple, cost effective and independent technique for the identification of the SLN in a patient diagnosed of breast cancer with a clinically negative axilla.

On the other hand, Isosulfan Blue and patent blue are structural derivatives of sulfonic acids which can combine with the amino acid group present on the protein surface, thereby having high protein binding capacity [16] predisposing them to allergic reactions and possible life threatening anaphylaxis [23]. At normal body temperature of 37°C, Methylene Blue shows no affinity to proteins [16].

Although the Radioisotopes used have been proved to be safe due to low levels of radiation, care must be taken during procedures involving either a pregnant lady or a pregnant surgeon [24,25], while Methylene Blue dye is the safest in pregnancy [26].

The optimal site of administration of the dye has been controversial. Mudun's study have demonstrated that the intra-dermal peri-areolar injection has a superior Identification Rate (IR) than the peri-tumoral and sub-dermal injections [15]. However, a study conducted by Rodeir showed similar IR in both the peri-areolar as well as peri-tumoral groups [27]. Our study compared the IR of the two groups using the Methylene Blue dye and showed significant superiority in the Peri-areolar group when compared to the Peri-tumoral group. Our results can be substantiated with the results of a study conducted by Ogasawara which demonstrated lymphatic drainage pathway using Indocyanine green fluorescence from the peri-areolar region detected in 33 of the 37 patients while only 12 of the 33 patients had a lymphatic drainage from the peri-tumoral region [14].

## Conclusion

Sentinel Lymph Node biopsy plays a vital role in the overall management of breast cancer with limited postoperative morbidity

and duration of hospitalization when compared to Axillary Lymph Node Dissection. Methylene Blue is a cost effective alternative to the Radioisotope technique with high accuracy, low false negativity and a high PPV. Methylene Blue dye is much safer and easily available when compared to Isosulfan Blue, Patent Blue and Indocyanine Green. Hence, Methylene Blue can be used safely in all patients.

The site of administration of the dye plays a vital role in intra-operative identification and the Peri-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.

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