

The sensitivity and specificity of methylene blue dye as a single agent in sentinel lymph node biopsy for early breast cancer

Aimir Ma'rof, MS¹, Nasser Muhammad Amjad, FRCS², Rokayah Julaihi, MS¹, Mark Hardin, MS¹, Sohail Mushtaq, FRCS³

¹Department of Surgery, Sarawak General Hospital, Kuching, Sarawak, Malaysia, ²Department of Surgery, Kulliyah of Medicine, International Islamic University Malaysia, Kuantan, Pahang, Malaysia, ³Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, Kota Samarahan, Sarawak, Malaysia

ABSTRACT

Introduction: Axillary lymph node dissection (ALND), although associated with significant morbidity, has been the standard procedure for axillary staging for breast cancer in many hospitals in Malaysia. The limited resources for radioisotope tracer and nuclear medicine service, coupled with insufficient number of trained surgeons, have been the major obstacles to perform sentinel lymph node biopsy (SLNB).

Materials and Methods: This study looks into the application of 1% methylene blue dye (MBD) as a single agent for SLNB and observes the outcome and any associated complication. Thirty-four patients with early breast cancer were enrolled. Two millilitres (ml) of 1% MBD was diluted with saline to a total volume of 5 ml. After induction of general anaesthesia, 3 ml of the diluted 1% MBD is injected subdermally at the upper outer quadrant of the breast followed by 5 minutes of massage. Sentinel nodes are identified as blue nodes or lymph nodes with a blue-stained lymphatic channel and were surgically removed. All patients then underwent tumour excision, either mastectomy or breast-conserving surgery, and ALND. The sentinel nodes were categorized to positive or negative for metastases and were compared with axillary lymph nodes for diagnostic value assessment.

Results: Identification rate of sentinel nodes was 91.2%. The mean number of removed sentinel nodes was 2 (SD=1) and the mean number of axillary nodes was 16 (SD=6). Sentinel node metastasis was found in 13 (41.9%) cases. There were two false-negative cases, resulting in a sensitivity of 86.7% (95%CI: 62.1-96.3). The negative predictive value of sentinel nodes to predict axillary metastasis was 88.9% (95%CI: 67.2-96.9). There were no complications observed.

Conclusion: Although inferior to the standard dual-tracer technique, the usage of MBD as a single agent in SLNB for early breast cancer still offers favourable accuracy and identification rate. With continuous training and improved surgeons experience, performing SLNB with blue dye alone is feasible in order to reduce the risks and morbidities associated with ALND.

KEYWORDS:

Breast cancer, sentinel lymph node biopsy, dye, sensitivity and specificity

INTRODUCTION

Breast cancer is one of the most common causes of cancer-related death worldwide. In 2012, 1.7 million cases of breast cancer were estimated by World Health Organization (WHO).¹ It accounts 25.1% of all cancers with standardized incidence rate of 43.1 per 100,000 and standardized mortality rate of 12.9 per 100,000.² In Malaysia, approximately 1 in 20 women will develop breast cancer in their lifetime with a higher incidence reported among Chinese followed by the Indians and the Malays.³

Over the years, the surgical treatment of breast cancer has developed substantially. It is known that the recurrence and survival rate in breast cancer are strongly dependent on the presence and extent of axillary lymph node involvement.⁴ Breast-conserving surgery for early breast cancer has reduced the major morbidity of mastectomy and therefore, greater consideration is now placed on the method for axillary staging.⁵ Conventionally, axillary lymph node dissection (ALND) is performed even for early breast cancer based on earlier reported evidence that it improves survival and reduces risk of recurrence.^{6,7} However, ALND is associated with significant morbidity such as lymphoedema, numbness, limited mobility, stiffness, and seroma formation, as well as risk for vascular and brachial plexus injury.^{4,8}

Many studies have proved that performing ALND for early breast cancer patients with clinically negative axillary nodes does not offer added benefit apart from subjecting patients for unnecessary morbidity and complication.^{4,9-12} For that reason, sentinel lymph node biopsy (SLNB) has been performed and studied over the years as an alternative to ALND. Current literatures support the use of dual-tracer technique for lymphatic mapping in SLNB, using both radioisotope and blue dye, as it results in the highest identification rates of up to 90% to 98%.^{13,14} On the other hand, the success rates for mapping with blue dye alone are slightly lower at between 83% and 93%.¹³

However, the non-availability of nuclear medicine service in many hospitals in Malaysia, coupled with limited resources for pre-operative lymphoscintigraphy and intra-operative radioisotope tracer have contributed to the difficulty in administering SLNB in Malaysia. Over the years, studies have been conducted to explore the possibility of performing SLNB using blue dye alone as a single agent. Few randomized

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Corresponding Author: Aimir Ma'rof

Email: aimir711@yahoo.com

studies showed that surgeons achieved equal results with favourable success rates when using blue dye alone compared with a combination of blue dye and radiolabelled colloid.¹⁵ Due to its cheap cost, methylene blue dye (MBD) was more extensively studied for SLNB, especially in developing countries.^{13,16-19} As the dye is readily available and affordable in Malaysia, this study aims to evaluate its sensitivity and specificity as a single agent in SLNB for early breast cancer.

MATERIALS AND METHODS

Study design

This cross-sectional study was conducted at Sarawak General Hospital, Kuching from January 2018 to June 2019. Convenient sampling was used to include all patients with early breast cancer who were admitted for an elective surgery.

Inclusion criteria

All patients diagnosed with breast cancer with tumour size of 3.0 cm or less, and negative ultrasound finding of axillary lymph nodes.

Exclusion criteria

Patients with no palpable breast lump, clinically palpable axillary lymph nodes, locally advanced tumour or large tumour more than 3.0 cm, inflammatory breast cancer, previous extensive surgery to the breast or axilla, previous neoadjuvant therapy, previous radiation therapy to the breast or axilla, pregnancy, prior history of allergy to blue dye, and patients who do not consent to be included in the study.

Sample size

This study aims to determine the sensitivity and specificity of MBD in detecting sentinel lymph nodes for biopsy. The prevalence of positive lymph node detection is estimated not less than 80%. Therefore, a minimum sample size of 39 subjects (including 31 subjects having the positive lymph node identification) will be required to achieve a minimum power of 80% (actual power = 80.7%) in order to detect a change in the percentage value of sensitivity from 0.70 to 0.90, based on a target significance level of 0.05 (actual = 0.048).²⁰

Study tool and protocol

Demographic data were collected from the medical notes and interviews with the patients. Clinical data were obtained from the medical notes, mammogram and ultrasound reports, and the histopathology reports.

General surgeons with more than 5 years of experience in breast cancer surgery and have undergone training to perform SLNB participated in the study. Early breast cancer defines as stage I and IIA, or tumour size of T1 and T2 with negative regional lymph node metastasis.^{15,21} However, for this study, we included only those with tumour size of less than 3.0 cm.^{10,11}

SLNB was performed using a 1% MBD (LaboratoiresSterop, Belgium). Two millilitres (ml) of 1% MBD was diluted with saline to a total volume of 5 ml. After induction of general anaesthesia, 3 ml of the diluted 1% MBD was injected subdermally at the upper outer quadrant of the breast followed with 5 minutes of massage.^{5,22} SLNB was then performed through a separate incision at the axilla. Sentinel nodes were identified as blue nodes or lymph nodes with a blue-stained lymphatic channel.²³ All patients subsequently underwent the planned surgery of either breast-conserving surgery and axillary clearance, or, mastectomy and axillary clearance.

The tumours, sentinel nodes, and the axillary lymph nodes were labelled separately. All specimens were fixed in formalin and sent for histopathological examination. The tumours were histologically classified according to the World Health Organization (WHO) Histological Classification of Breast Tumours.¹ Molecular subtypes were classified as luminal A (ER+ and/or PR+, HER2-, and histological grade either 1 or 2), luminal B (ER+ and/or PR+, HER2+; ER+ and/or PR+, HER2-, and histological grade 3), HER2 positive (ER-, PR-, HER2+), and triple negative (ER-, PR-, HER2-).^{24,25} The sentinel nodes were categorized as positive or negative for metastases based on the histological evidence of metastatic carcinoma. The findings were compared with axillary lymph nodes for diagnostic value assessments.

Statistical analysis

The categorical data were presented as frequency and percentage. The numerical data were presented as mean and standard deviation in a normally distributed data, while for not normally distributed data, they were presented as median and interquartile range.

Analysis of the data was performed at 95% confidence interval (CI) using SPSS version 23.0 and OpenEpi online calculator (openepi.com). The false-negative rate was determined as false negative/(true positive + false negative). Sensitivity was measured as true positive/(true positive + false negative) while specificity as true negative/(true negative + false positive). The negative predictive value was calculated as true negative/(true negative + false negative) while the positive predictive value as true positive/(true positive + false positive).

Safety and ethical consideration

This study was approved by the National Medical Research and Ethics Committee (MREC) of the Ministry of Health, Malaysia with KKM/NIHSEC/P17-1694(16) and KKM/NIHSEC/P17-1694(18) as reference. All patients were given a patient information sheet and written informed consents were obtained.

The safety of patients was ensured and potential complications associated with the usage of blue dye such as allergic reaction and skin necrosis were constantly observed and monitored. The privacy and confidentiality of patients were always protected, and all data and personal information were kept classified.

Table I: Demographic and clinical characteristics of patients (n=34)

Characteristics	Value
Age, years old, mean (SD)	53 (11)
Race, n (%)	
Malay	14 (41.2)
Chinese	10 (29.4)
Iban	6 (17.6)
Bidayuh	4 (11.8)
Diameter of tumour, n (%)	
< 2.0 cm	17 (50.0)
2.0–3.0 cm	17 (50.0)
Site of tumour, n (%)	
Upper outer quadrant	20 (58.8)
Lower outer quadrant	8 (23.5)
Upper inner quadrant	5 (14.7)
Lower inner quadrant	1 (2.9)
Histology type, n (%)	
Invasive carcinoma of no special type	33 (97.1)
Medullary carcinoma	1 (2.9)
Molecular subtypes, n (%)	
Luminal A	16 (47.1)
Luminal B	11 (32.4)
HER2 positive	2 (5.9)
Triple negative	5 (14.7)
Surgery, n (%)	
Breast conserving surgery	16 (47.1)
Mastectomy	18 (52.9)

Table II: Identification of sentinel and axillary lymph nodes metastases (n=31)

	Axillary lymph nodes metastases	
	Yes	No
Sentinel lymph nodes metastases		
Yes	13	0
No	2	16

Table III: Diagnostic value of methylene blue dye in sentinel lymph node biopsy

	Value	95%CI
Sensitivity	86.7%	62.1 - 96.3
Specificity	100.0%	80.6 - 100.0
Positive predictive value	100.0%	77.2 - 100.0
Negative predictive value	88.9%	67.2 - 96.9

RESULTS

A total of 34 patients were included in the study with a response rate of 87.2% (34/39). The demographic and clinical characteristics of the patients are summarized in Table I.

The mean age of patients was 53 years old (SD=11) and the ethnic composition of the respondents was 41.2% Malay, 29.4% Chinese, 17.6% Iban, and 11.8% Bidayuh. Majority of the tumours were located at the upper outer quadrant of the breast (58.8%) and there was equal distribution in term of tumour size. Almost all the tumours were reported histologically as invasive carcinoma of no special type (97.1%) and luminal A was the most common molecular subtypes (47.1%). 18 patients (52.9%) underwent mastectomy while 16 patients (47.1%) had a breast-conserving surgery.

The study identified sentinel lymph node in 31 patients and therefore, the identification rate was 91.2%. The mean number of removed sentinel lymph nodes was 2 (SD=1) and the mean number of axillary lymph nodes was 16 (SD=6).

Among the 31 patients with identified sentinel lymph nodes, 13 of them (41.9%) were reported as positive for metastasis as shown in Table II. There were two false-negative cases and the false negative rate was calculated as 13.3%. As listed in Table III, the sensitivity of MBD to identify the sentinel lymph nodes was 86.7% (95%CI: 62.1, 96.3) with a negative predictive value of 88.9% (95%CI: 67.2, 96.9).

There were no allergic reaction or local inflammatory reaction at the injection site such as skin necrosis or ulceration. All patients had an uneventful post-operative recovery and were discharged well.

DISCUSSION

SLNB is considered the gold-standard treatment and has replaced ALND in the evaluation of axillary staging for early breast cancer. Despite the knowledge that ALND is associated with many risks and morbidities,^{4,8,26} SLNB has not become a common practice among surgeons in Malaysia. Firstly, majority of patients with breast cancer in this country present late to the hospital, either in a locally advanced or advanced stage.³ The other factors include the non-availability of nuclear medicine service in many hospitals in Malaysia, as well as the limited financial resources for the radioisotope tracer. In addition to that, many surgeons are not trained and credentialed to perform SLNB.

Several other developing countries like Malaysia are facing similar problems and therefore, studies have been conducted over the years to look at the possibility of performing SLNB using blue dye alone as a single agent. There have been many promising results with favourable identification rates and low false-negative rates.^{13,18,22}

The response rate of this study was 87.2% as it did not achieve the target sample size of 39. However, the positive identification of sentinel lymph nodes in 31 patient fulfils the requirement of the study to achieve a minimum power of 80% for data analysis. The most obvious factor was the difficulty in recruiting patient with early breast cancer in the study hospital. Majority of breast cancer patients present late at a locally advanced or advanced stage. The effort to educate the public on awareness of screening and early detection should be continued and carried out on a frequent basis.

The mean age of patients was 53 years old with the youngest at 36 and the oldest at 73. Malay ethnicity composed the majority of patients in this study, followed by Chinese, Iban, and Bidayuh. Although previous data showed a higher prevalence of breast cancer among Chinese in Malaysia,³ this difference might be due to higher Malay population (61.8%) compared to Chinese (37.3%) in Kuching, Sarawak which was the location of the study hospital.²⁷

Consistent with other epidemiological data,²⁷ this study showed that the commonest breast cancer is the invasive carcinoma of no special type (97.1%) and the main molecular subtype is luminal A (47.1%). Sixteen patients (47.1%) underwent mastectomy while the other 18 (52.9%) opted for breast-conserving surgery. However, the decision for type of surgery was not made based on the size and the location of tumour. Rather, it was decided by the patients and the attending surgeons after the surgical options and subsequent treatment plan were explained in detail.

In overall, only 15 patients (44.1%) were lymph node positive for metastases. It means that more than half of them could have been spared from the risks and morbidities of ALND. This finding is consistent with other previous reports^{4,9-12} and further accentuates the benefit of SLNB for early breast cancer.

The sentinel lymph nodes were identified in 31 patients, resulting in identification rates of 91.2%. It is lower than the standard dual-tracer technique with both radio labelled

colloid and blue dye.^{13,14} However, it is comparable with other similar studies that used MBD as a single technique in SLNB.^{17-19,22} Most importantly, it surpasses the recommendation by the American Society of Breast Surgeons which stated that the sentinel lymph node identification should be above 85% in order to abandon axillary dissection.¹⁵

The age of the three patients with unidentified lymph nodes was 36, 65, and 66 years old, respectively. All three of them had tumour size of <2.0 cm with negative axillary lymph node metastases. Many factors were reported to be associated with the success or failure in identifying sentinel lymph nodes which include age, body mass index, tumour size, grade and location, as well as SLNB technique and surgeons experience. This study did not look at these associated factors in detail but the older age, small tumour size, and limited surgeons experience were in tandem with the findings from some of the studies.²⁹⁻³¹

There were two patients with false-negative outcome, resulting in false-negative rates of 13.3%. It is higher than the average rates of 8.4%, and the recommendation by American Society of Breast Surgeons on acceptable false-negative rates to abandon axillary dissection should be 5% or less.¹⁵ False-negative results are usually down to lack of experience and technical failures which may be avoidable.¹⁴

There are few possible reasons that this study did not achieve the recommended 5% false-negative rates. The numbers of sentinel lymph node harvested is one of the factors that influence the false-negative rates.³²⁻³⁴ The mean number of two sentinel lymph nodes obtained in this study could contribute to the final result. Although it fulfils the minimum number of sentinel lymph nodes required for SLNB in breast cancer as suggested by other studies, they showed that the false-negative rates significantly decrease as the number of lymph nodes rises.³²⁻³⁴ Hence, removing more than two sentinel lymph nodes in subsequent study or clinical practice may reduce the false-negative rates.

In both cases with false-negative result, the tumour was <2.0 cm in size and located at the lower outer quadrant. A previous study contradicts this observation, as they reported a significant increase in the false-negative rates for tumours located in the upper outer quadrant compared with other locations.³¹ Surgeons' performance could also factor in the result. General surgeons involved in this study, although proficient in breast cancer surgery, have limited experience in SLNB. However, it has been reported that performing SLNB using blue dye demonstrates a short learning curve among experienced surgeons. Surgeons are advised to perform at least 20-30 cases before they can achieve the highest identification rate and lowest false-negative rates.^{15,16}

As a result, the sensitivity and negative predictive value (NPV) in this study were 86.7% and 88.9%, respectively. It is lower compared to other studies that reported sensitivity as high as 91.7% to 94.0% and NPV as high as 90.0% to 96.1%.^{22,35} A better outcome can be achieved in future by increasing the identification rates and reducing the false-negative rates.

There was no incidence of severe anaphylactic reaction in all patients. Although previous studies reported local inflammatory reaction with MBD, no complications were observed in this study. This could be due to the usage of diluted dye as recommended by previous literatures.^{5,13,17}

The lower sensitivity and specificity of MBD as a single agent for SLNB shown in this study proves that the dual-tracer technique with radio labelled colloid and blue dye remains the gold standard in current clinical practice. However, in places with limited resources and expertise, this study has shown that performing SLNB with blue dye alone has favourable accuracy and identification rate to reduce the morbidity of ALND. Future studies and training should be designed to involve more general surgeons in the hope that SLNB will be practiced widely in future for the benefits of the patients.

CONCLUSION

Although inferior to the standard dual-tracer technique, the usage of MBD as a single agent in SLNB for early breast cancer still offers favourable accuracy and identification rate. With continuous training and improved surgeons experience, performing SLNB with blue dye alone is feasible in order to reduce the risks and morbidities associated with ALND.

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DISCLOSURE

None.

CONFLICT OF INTEREST

None.

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