

Frozen Section Evaluation of Methylene Blue Dye based Sentinel Lymph Node Biopsy in Early Breast Cancer – A Feasibility Pilot Study in a Tertiary Cancer Centre

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ABSTRACT

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Background: Most of the major cancer centres have adopted sentinel lymph node biopsy (SNLB) as standard means of axillary nodal assessment as an alternative to axillary lymph node dissection (ALND) in clinically node negative early breast cancers. The benefits of SNLB is to reduce the functional disability which is a potential consequence of ALND. A major pitfall of SNLB is the potential of false negative rate which will increase the risk of an axillary recurrence.

Methods: This is a pilot study of forty one cases of clinically N0, T1 and T2 tumors for whom sentinel lymph node mapping was done using methylene blue dye to detect occult metastasis. This was validated by complete ALND in all cases.

Results: The calculated accuracy of the frozen section analysis was 97.5%, sensitivity was 85.7% and specificity was 100%. Positive predictive value was 100%, negative predictive value was 97.14% and false negative rate was 2.86%

Discussion & Conclusion: This study demonstrates a high sensitivity and specificity for frozen sections of SNLB with an accuracy of 96%.

Keywords: Frozen section, Sentinel lymph node, Methylene blue dye, Early breast cancer

*See End Note for complete author details

BACKGROUND & RATIONALE

The status of axillary lymph node is considered an important prognostic factor in the management of breast cancer. Traditionally axillary lymph node dissection (ALND) was performed as an integral part of breast cancer surgery for purposes of staging, to obtain regional control and to decide adjuvant treatment. However, ALND is associated with a recognized short and long term side effects including the risk of paresthesiae or pain, hematoma, seroma, restricted shoulder movement and lymphedema¹. The only patients who can derive benefit from axillary dissection are those with positive nodes, accounting for 40% or fewer of all patients who undergo ALND. Removal of healthy lymph nodes offers no benefit outcomes. This leads to the necessity of a more selective approach to managing the axilla, namely the sentinel lymph node biopsy (SLNB). The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-32 trial was designed to determine whether sentinel node resection achieves

the same therapeutic outcomes as axillary dissection but with fewer side effects⁶. The study concluded that the overall survival, disease free survival, and regional control between the two treatment groups were not statistically significant.

Sentinel lymph node biopsy based on dual dye technique, based on combined radiocolloid and isosulphan blue dye is the standard of care for the assessment of clinically negative axillary lymph nodes in patients with early breast carcinomas⁷. However the non-availability of nuclear medicine facility and the high cost even when available had stood in the way of many institutions, especially in developing nations, in adopting this technique. Blue dye alone based sentinel node biopsy technique is simple, fast and less taxing in terms of resource utilization. Several authors have described experience with this technique predominantly from developing nations. In this article we evaluated the feasibility of blue dye based sentinel node biopsy and its role in clinical practice in a tertiary cancer centre.

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OBJECTIVES

The aim of the study is to evaluate the accuracy, sensitivity and specificity of frozen section in the detection of metastasis in axillary sentinel lymph nodes of early breast cancer patients

MATERIALS AND METHODS

Prior approval was obtained from institutional scientific review board and from hospital ethics committee.

During the study period, forty one patients who fit in the inclusion criteria of clinically N0, T1 and T2 tumors for whom sentinel lymph node mapping was done, were selected. Tumor size of T3 and node positive disease were excluded. Mode of surgery, whether breast conservation or modified radical mastectomy was based on the patient preference and the decision in multispecialty tumour board. The technique of sentinel node mapping involves injecting 6 ml of sterilised methylene blue into the retro areolar plane followed by massaging the skin for five minutes; after 20 minutes the axilla was explored and the first draining lymph node/nodes which showed the blue color was dissected out and subjected for frozen section to look for metastasis. The freshly harvested and dyed lymph node were bisected, frozen sections cut at a thickness of 4-6 microns in a Leica cryostat and stained with Haematoxylin & Eosin. Two to three frozen sections were studied for each node. The sections were examined for macrometastasis (>2mm) or micrometastasis (<2mm). The results were communicated with a turn-around time of 20-30 minutes. The remaining tissues were embedded in paraffin for permanent sections to be examined at an extra level. Later a routine surgical procedure (BCS or MRM) with complete axillary clearance (ALND) was done for all cases irrespective of the results of sentinel lymph node examination by frozen section. Histopathological examination of the whole surgical specimen comprising breast and axillary lymph node dissection was performed.

Post operative adjuvant treatment (chemotherapy, radiotherapy or hormonal therapy) was given to the patients if needed, according to the standard protocol of the institution.

The statistical calculations of accuracy, sensitivity, specificity, positive & negative predictive values were done on the data obtained.

RESULTS

The median age of our patients was 52 years within a range of 32 to 74 years.

Total 1. Results of frozen section analysis

Frozen section of sentinel nodes	Histopathology of sentinel nodes		
	Positive	Negative	Total
Positive	6	0	6
Negative	1	34	35
Total	7	34	41

Total 2. True positive SLN cases [n=6] and its ALND

Sl. No	Age	No of SLN positive/dissected	No of ALN positive / dissected
1	49	1/3	0/17
2	35	1/3	1/19
3	40	1/2	1/17
4	32	1/1	4/17
5	54	1/2	0/13
6	47	1/1	0/23

- Sentinel lymph nodes were identified in all 41 cases.
- The number of sentinel lymph nodes harvested ranged from one to seven, with an average of two in the majority.
- Out of 41 cases, 35 showed negative results on frozen section, implying no metastasis was detected and six showed positive results, indicating presence of metastasis.
- Of the 35 negative results, 34 were true negative (97.14 %) when matched with the corresponding paraffin sections and one was false negative, implying that metastasis was missed on frozen section but detected on the paraffin section.
- Of the six positive cases, all were true positive (100%) implying that metastasis detected on frozen was confirmed on paraffin section also. There was no case of false positive (**table 1**).
- 16 out of 41 cases (39%) showed positive ALND, of which SLNB was positive in three. The remaining three cases of positive SLNB did not have positive ALND (**table 2**).

The calculated accuracy of the frozen section analysis was 97.5%, sensitivity was 85.7% and specificity was 100%. Positive predictive value was 100%, negative predictive value was 97.14% and false negative rate was 2.86%

DISCUSSION

For decades, axillary lymph node dissection (ALND) has been the standard procedure in surgical treatment of patients with breast cancer. Breast screening programmes and heightened public awareness of the disease have led to smaller tumour size at presenta-

tion and a lower proportion of patients with nodal involvement. Only upto 40% of patients with clinically negative axilla have occult disease in the axilla. For the rest of the patients axillary dissection is unnecessary and they suffer from the morbidity associated with ALND. Women who undergo axillary dissection carry risk of developing multiple short term and long term complications in the arm and psychological distress.¹⁻³ Among the long term complications, lymphedema is one among the commonest. Lymphedema can cause limitations in range of motion, pain, weakness or stiffness in the affected extremity. Literature reports a broad range of incidence for post-operative lymphedema, varying widely from 6% to 56% in various ALND patients, depending on definition, method of measurement, extent of axillary surgery, number of different surgeons, choice of adjuvant therapy and time elapsed since operation.

A potential alternative to axillary lymphadenectomy is sentinel node resection. Sentinel lymph nodes are more likely to have metastatic disease as compared to non sentinel lymph nodes; likewise occult metastasis is more likely to be identified in deeper paraffin sections in sentinel lymph nodes.⁴ Many studies are available in the literature comparing ALND and SLNB. Preliminary studies have shown that SLNB in breast cancer populations, compared to standard ALND, has a sensitivity varying from 85% to 98%. The problems in sentinel lymph node evaluation are due to the lack of standardised classification criteria, standard protocols for node evaluation, and limited clinical outcome data.

The methylene blue based SLNB technique was first described by Giuliano et al.⁵ It is cheap and readily available. As the false negativity rate approached 14% in early series, blue dye alone based SLNB fell out of favor in the West. The sentinel lymph node technique based on dual dye requires gamma camera and nuclear medicine facility which is not available in many institutions especially in developing nation. Even in institutions with the facility, overcrowding and high cost have restricted their routine usage. For this reason SLNB has not been adopted in several institutions in the developing nations. In this scenario several oncology practitioners have published their experience with SLNB using blue dye alone. However the published results of this technique are mixed. The negative predictive value and false negativity varies across studies.⁶⁻¹⁰

Injection of tracer like vital blue dye is a common technique of identification used. The route of tracer administration can be either the skin (over the primary tumour site) or the primary tumour itself. The skin sites can be peri-areolar, sub-areolar, intra-dermal or subcu-

taneous. Intradermal injection of tracer dye is known to be successful, which acknowledges the fact that both dermal and parenchymal lymph nodes drain to a common SLN. The tumour sites can be either peritumoral or intra-tumoral.

Techniques of radioisotope scanning with the use of a hand held gamma probe is an alternative. Evidence from peer-reviewed pilot studies on using blue dye, radioisotope or a combination of both suggests that very high identification rates can be reached by the combination rather than single agent mapping.¹¹ The study concluded that identifying multiple sentinel lymph nodes rather than a single node reduced the false negative rate. With increase in patient age, difficulty was noted in detection of sentinel lymph node with fewer nodes being picked up.

The CAP and ASCO recommended practise of slicing a sentinel lymph node at intervals of two mm parallel to the long axis and examining one H & E stained section from the each slice which should include a full cross-section of the slice including the capsule and subcapsular space can detect all metastasis larger than 2mm.⁶ Smaller or micrometastasis could still be missed and isolated tumor cells (ITC) are found in the SLNB on immunohistochemistry. Thus immunohistochemistry on frozen section provides valuable information that can lead to fewer secondary ALNDs.¹² The American College of Surgeons Oncology Group (ACOSOG) study Z0010, a prospective multicentre study of over 5000 patients with a eight year follow up concluded that where occult metastasis was detected by additional haematoxylin and eosin step-level sections and/or immunohistochemical staining for cytokeratins in initially negative SLNB, offered no significant improvement on survival. Hence any protocol that suggests more serial sections to be taken with immunohistochemistry back up, must be considered as experimental till validated with information on the prognostic significance of micrometastasis or isolated tumor cells (ITC).¹³

The current American Society of Clinical Oncology (ASCO) and National Comprehensive Cancer Network (NCCN) recommendations regarding the role of complete ALND for patients undergoing SLNB says that completion ALND is not indicated in patients with negative SLNB including histologic evidence of ITC. If there are more than three pathologically involved sentinel nodes, a completion ALND is done for staging and local control. For patients with one to three involved lymph nodes or micrometastasis, they can be offered choice of regional radiotherapy to ALND.^{14,15}

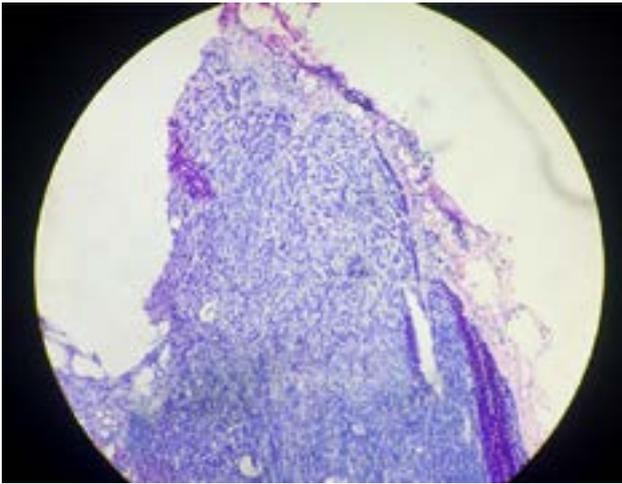


Figure 1. H & E (100X) of FFPE section showing axillary LN with diffuse metastatic infiltrate

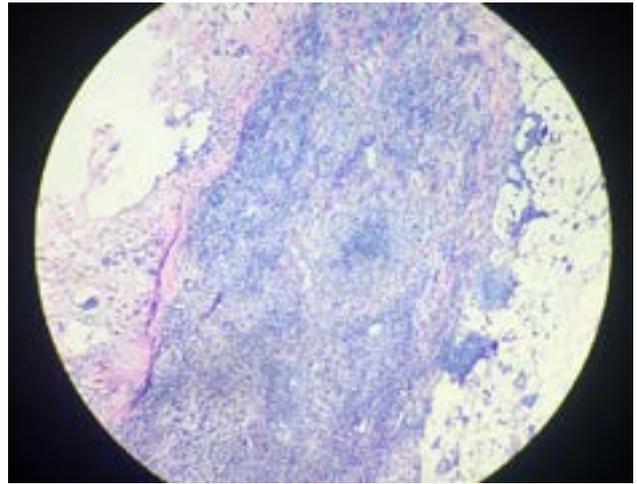


Figure 2. H & E (100X) Frozen section of sentinel lymph node showing metastatic deposits

Our study validated the results of forty one cases of intra-operative frozen section analysis of sentinel lymph nodes with the results of permanent sections of the same as well as those of the rest of axillary lymph nodes obtained in ALND (**figure 1**). The tracer dye used was methylene blue and the average yield of sentinel lymph nodes were two to three. The results showed a sensitivity of 85.71%, specificity of 100% and accuracy of 96.88% (**figure 2**). This data correlates with other studies where reported sensitivity and accuracy were in the range of and 54-94% and 84-95% respectively.^{11,13,16,17} Almost all studies on sentinel lymph node report a finite number of false negative cases. The single case of false negative in this study was attributed to the limited number of frozen sections taken as the metastatic focus was picked upon taking deeper sections of the corresponding permanent paraffin block.

Of the 41 cases, 16 were positive for metastasis in the final ALND. The range of positive nodes ranged from one to six; half the number of cases showing single node positive for metastasis. Among these 16 cases, SLNB was negative in thirteen. In these 13 cases the yield of sentinel nodes picked was only one in half of the patients. This is a described pitfall of SLNB where the yield of blue staining nodes could be low either due to technical errors or aberrant mammary lymphatics.

The American College of Surgeons Oncology Group (ACOSOG) study Z0010,^a a prospective multicentre study of over 5000 patients with a eight year follow up concluded that where occult metastasis was detected by additional haematoxylin and eosin step-level sections and/or immunohistochemical staining for cytokeratins in initially negative SLNB, offered no significant improvement on survival. Prospective analysis of whether axillary recurrences occurred in patients with micro-

metastasis in SLNB, in whom a formal ALND was performed, showed that after a median follow-up of 42 months there was no evidence that presence of micrometastasis leads to axillary recurrence or distant disease.^{7,18,19}

An alternative is do an axillary sampling where a minimum of four to six suspicious nodes are evaluated intraoperatively with frozen section.^{20,21} Use of blue dye guided sampling can potentially improve the results.^{22,23} This method of axillary sampling has a comparable accuracy as the dual dye based SLNB.²¹

The NSABP B-32 trial which compared SLNB followed by ALND versus SLNB followed by ALND only if SLN was positive, found a false negative rate of 9.8 %. There were no significant differences in terms of regional control, overall survival or disease free survival between the groups at a median follow up of eight years.^{14,24}

A point to note is that the necessity of complete ALND in cases with SLN positivity limited to one or two lymph nodes has been the focus of recent clinical trials with the results in favour of safely omitting ALND in clinically N0, with metastasis in upto two SLN. A study conducted by The American College of Surgeons Oncology Group (ACOSOG) was a prospective randomised trial to probe the benefit of additional ALND in invasive carcinomas, namely c T1-T2, N0 with H & E detected metastasis in one or two SLN treated with breast conservation surgery followed by radiotherapy. They found no significant differences in five year overall or disease free survival and the rate of recurrence.²⁵ Thus the findings of recent clinical trials have resulted in fewer ALNDs in cN0, SLN positive patients.

CONCLUSION

The goal of all SLNB is to detect all macrometastasis (>2mm) as well as micrometastasis (< 2mm). Our pilot study has given results comparable to related studies in terms of sensitivity, specificity and accuracy of frozen sections. Hence we conclude that frozen section analysis of SLNB is a reliable tool to decide the need for further ALND and the latter can be avoided in cases with negative sentinel nodes and even in positives upto two nodes.

LIMITATION

The yield of sentinel lymph nodes were suboptimal in a few cases. This could account for the positivity for metastasis in ALND inspite of the negative sentinel node biopsy.

END NOTE

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Abbreviations

SNLB - Sentinel Lymph Node Biopsy
ALND - Axillary Lymph Node Dissection
ACOSOG - The American College of Surgeons
Oncology Group

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Editor's Remarks: The management of breast cancer has evolved over years with better results in survival. This original research deals with the detection of metastasis in the sentinel axillary lymph nodes using frozen section biopsy techniques in an accurate, sensitive and dependable manner. Worth reading for the original work done.

Conflict of Interest: None declared

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