

## ROLE OF INTRAOPERATIVE IMPRINT CYTOLOGY FOR DIAGNOSIS OF SENTINEL NODE METASTASIS IN BREAST CANCER- A TWO YEAR PROSPECTIVE STUDY IN A TERTIARY CARE CENTRE

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### ABSTRACT

#### BACKGROUND

Sentinel node imprint cytology has emerged as a standard diagnostic modality for breast cancer. It represents a reliable predictor of axillary lymph node status in cancer patients. This study was undertaken to assess the diagnostic accuracy of imprint cytology as an intraoperative tool for evaluating sentinel node in clinically node negative breast cancer patients.

#### METHODS

This study was conducted in the Department of Pathology, VIMSAR, Burla from November 2016 to September 2018. Out of 60 newly diagnosed invasive mammary carcinoma cases, sentinel lymph node was identified intraoperatively in 52 cases by mapping with methylene blue and gentian violet. Subsequently, study was done by staining the imprinted slides of sectioned lymph node with Diff-Quick and Papanicolaou stain, later correlated with permanent histopathology study.

#### RESULTS

Out of a total of 52 patients, SLN metastasis was seen in 12 cases in intraoperative cytology but in permanent histopathology sections 20 were positive for metastasis. Methylene blue was 93-95% sensitive and 100% specific in detecting the sentinel node. Also, gentian violet which is not routinely used as a mapping dye, showed 95% sensitivity and 99% specificity.

#### CONCLUSIONS

This study demonstrates the accuracy of mapping and identification of sentinel lymph node by the blue dyes during surgery. It determines the therapeutic significance of rarely node positive case for metastasis which were clinically node negative.

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#### BACKGROUND

Worldwide, breast cancer is the most frequently diagnosed life threatening cancer in women, and the leading cause of cancer death. In India, the incidence of breast cancer is rising, and it comes next to cervical cancer so far.<sup>1</sup> Dread of cancer is rampant amongst the civilized world. Early detection is the way to lessen its impact on life. The single most important predictor of outcome for women with Breast carcinoma is the status of regional lymph nodes. Imprint cytology (touch preps) is a simple and inexpensive method of detecting metastatic disease in the SLN.<sup>2</sup> Sentinel lymph node mapping is attractive as it may identify a population of breast cancer patients that may benefit from axillary lymph node dissection.<sup>3</sup> The worldwide interest in Sentinel lymph node dissection (SLND) is understandable because this minimally invasive procedure may change the method of staging in a variety of solid tumours. Currently, intra-

operative evaluation performed by using intra-operative imprint cytology or intra-operative frozen sectioning imprint cytology (touch preps) is a simple and inexpensive method of detecting metastatic disease in the SLNs but has the disadvantage that the number of the cells examined is insufficient. At present, axillary lymph node dissection (ALND) remains the standard of care for surgical management of all patients with invasive breast carcinoma, as it provides precise staging and excellent local control. Currently, there is no standard approach to Sentinel lymph node dissection (SLND), which has made this technique even more challenging for the novice. In this section, we have discussed our experience of lymphatic mapping with the use of Blue dye imprint cytology at the VSS Institute of medical science and research (VIMSAR) Burla.

#### Aims and Objectives

This study was undertaken with an objective to assess the diagnostic accuracy of imprint cytology as an intra-operative tool for evaluating sentinel node in patient with clinically node negative breast cancer.

#### METHODS

A total no. of 82 sentinel lymph nodes were extracted from 52 patients undergoing breast surgery in Department of Surgery, VIMSAR Burla from November 2016 to October

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2018. Intra-operative evaluation of sentinel lymph node involvement is then compared with final Histopathological reports of permanent section.

**Inclusion Criteria**

- (1) Presence of palpable breast cancer;
- (2) Tumour size clinically equal to or less than 3 cm in diameter; and
- (3) Absence of clinically palpable axillary lymph nodes.

**Exclusion Criteria**

All high-grade breast carcinoma patients and node positive patients were excluded from this study.

**Surgical Protocol**

A surgical protocol was used to identify sentinel lymph node. In all cases peritumoral injections of methylene blue and gentian violet were used Intra-operatively to provide visual identification of the SLN and they are harvested by axillary dissection.

Dose: 1 ml of 0.1% methylene blue injected peritumorally.

**Methods**

After axillary dissection all the blue lymph nodes were extracted and immediately dissected longitudinally into two halves. The cut surfaces of lymph nodes are touched over clean glass slides holding the tissue in between thumb and index finger. Multiple touch smears were taken. The harvested lymph nodes were sent for permanent Histopathological examination.

**Pathological Examination**

All the touch smears were stained with Diff-Quick and subsequently Papanicolaou stain.

Imprints were reviewed intra-operatively by our team of pathologist with informal cytologic training.

Diagnostic categories included positive or negative for tumour or atypical cells present. Positive result prompted completion of the axillary lymphadenectomy, and a negative result no further lymphatic dissection. Completion of the lymphadenectomy was at the surgeon’s discretion due to presence of atypical cells. The surgical team was subsequently notified of the result. After an interpretation was rendered, the SLN was fixed in 10% formalin, processed in the usual manner, and embedded in paraffin. A single H & E stained section of the SLN was cut from the paraffin block and examined.

When discrepancies existed between the intra-operative and permanent section results, slides were again reviewed by an independent pathologist in an attempt to determine the cause of the discrepancy. When non-SLNs were obtained, they were examined using standard pathologic techniques. If greater than 4 mm in width, non-SLNs were sectioned; and if less than 4 mm, non-SLNs were submitted whole. Routinely, a single H&E stained section of the non-SLNs was examined and in several cases multiple levels were obtained in an attempt to verify the presence of metastasis.

Chi square and Fisher exact test (as appropriate) were used to assess differences in clinical and pathologic factors between those with and those without SLN performed and to assess the association between pathologic factors and Intraoperative cytology (IIC) results.

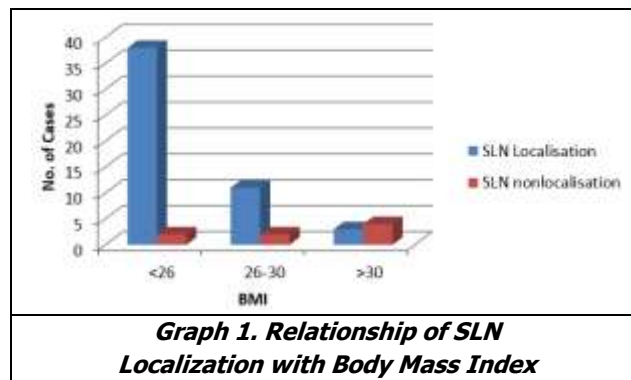
**RESULTS**

We attempted SLN mapping procedures in 60 consecutive patients and SLN was identified in 52 (87.2%) of these Patients. All 52 consecutive cases with intra-operative imprint cytologic diagnosis of the SLN were evaluated as per protocol. From this cohort of 52 patients, 5 cases (9.6%) were of pure invasive lobular carcinoma, 37 (71.2%) of pure invasive ductal carcinoma. These patients ranged in age from 21 to 87 years (mean, 60 years). Breast-conserving surgery was performed in 69% of patients and the remaining 31% underwent mastectomy.

Sometimes it was seen that BMI has a great effect on localization of lymph nodes. Hence BMI was calculated in all the 60 patients and grouped into three groups. Group-1(BMI below 26), group-2 (BMI in between 26 to 30), and group-3(more than 30). p-value of the study is less than 0.050 hence it is statistically significant. (Table 1, Graph 1)

BMI	SLN Localisation	SLN Non-Localisation
<26	38	02
26-30	11	02
>30	03	04

**Table 1. Visualization of Sentinel Lymph Nodes According to Body Mass Index**



Intra-operative success rate was correlated with size of lesion. Sentinel node extraction was successful in 52 cases. Out of which SLN metastasis was seen in 12 cases in intra-operative cytology but in permanent sections 20 were positive for metastasis among which 32 no. of patients were having mass size less than 3 cms. and 20 were greater than 3 cms. The overall sensitivity is 58.9% for patients less than 3 cms. and 62.5% in greater than 3 cms. (Table 2)

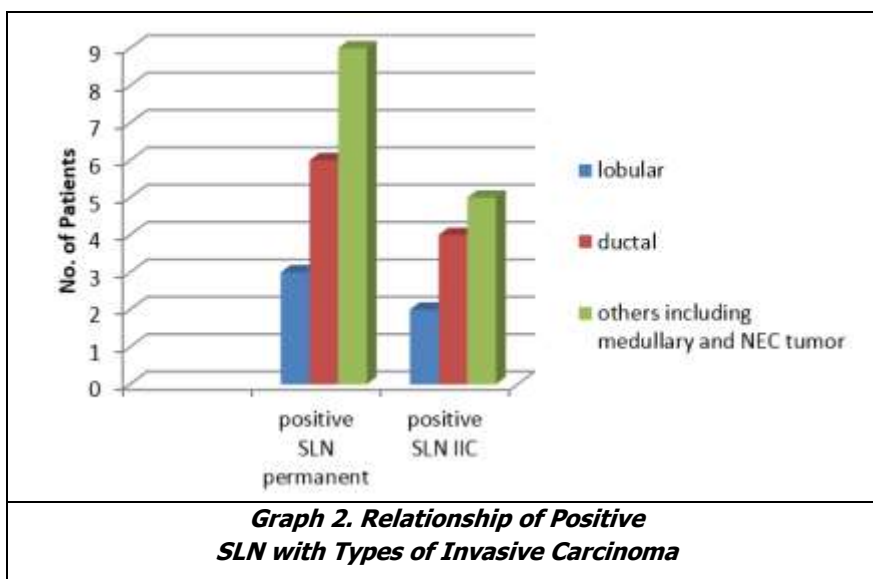
Patients with Mass Size	Positive IIC	Positive in Permanent H.P. Study	Sensitivity
<3 cm (32)	7	12	58.9%
>3 cm (20)	5	08	62.5%

**Table 2. Intra Operative Success Rate Correlation with Size of Lesion**

We encountered two types of invasive carcinoma of breast one is invasive ductal carcinoma NOS and invasive lobular carcinoma. Along with these two types we also had one case of medullary carcinoma of breast and one of neuroendocrine carcinoma of breast. Both of these tumours were negative for SLN metastasis and showed reactive lymphoid pattern. Also, we had two cases of metaplastic squamous cell carcinoma of breast out of which one showed metastatic SLN in IIC and confirmed by permanent H & E sections. Overall sensitivity of invasive lobular and ductal carcinoma is similar 66.7% and others to be 56%. (Table 3, Graph 2).

Types of Invasive Carcinoma	No. of Patient	Positive SLN Permanent	Positive SLN IIC	No. of False Positive IIC	Sensitivity	Specificity
Lobular	08	3	2	2	66.7%	100%
Ductal	37	6	4	4	66.7	100%
Others Including Medullary and NEC Tumour	15	9	5	1	55.6	100%

**Table 3. Sensitivity of Intra-Operative Diagnosis in Relation to Histological Typing of Invasive Carcinoma**



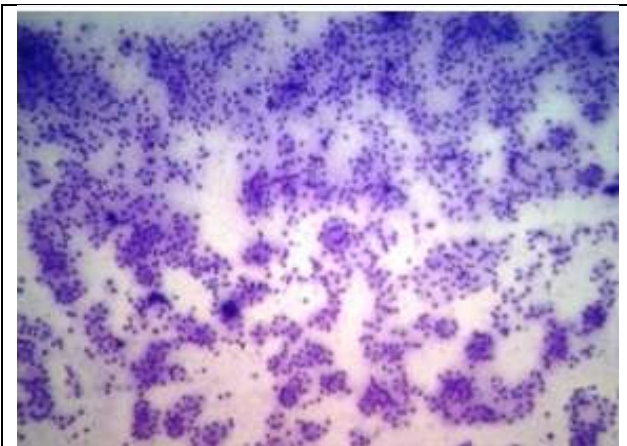
Effect of multiple slicing of lymph node was done for better detection of SLN metastasis in which out of 52 patients total 82 lymph nodes were extracted. 30 lymph nodes were bisected and rest 52 were sectioned into 4mm thick slices for better visualization of cellular details. The sensitivity of positive lymph nodes was 83.4% in multiple sliced nodes and 40% in bisected lymph nodes. (Table 4)

Method of Evaluation	No. of Lymph nodes	No. of +ve Permanent	No. of +ve IIC	Sensitivity	Specificity
Bisected	30	10	4	40%	100%
Multiple Slicing 5 mm	52	18	15	83.4%	100%

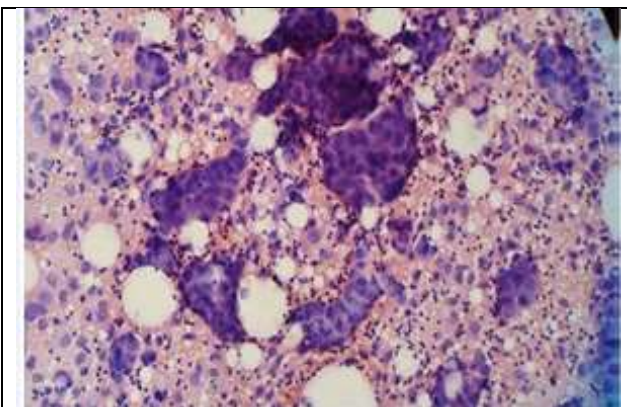
**Table 4. Effect of Multiple Slicing on Evaluation of Lymph Nodes**



**Figure 1. Blue Dye Stained Sentinel Lymph Node**



**Figure 2. Imprint Smears Showing Lymphoid Cells Without Any Tumour Cell (100x Diff Quik Stain)**



**Figure 3. Imprint Smears Showing Tumour Cells in Adeno Pattern (400x Diff Quik Stain)**

## DISCUSSION

It is well accepted that axillary node status is the most important prognostic indicator in patients with invasive breast cancer. Knowing the nodal status is essential for correct cancer staging, it helps determine the need for adjuvant therapies. However axillary lymph node dissection (ALND) is associated with significant morbidity, with up to 60% of women experiencing long-term side-effects. Moreover, ALND is unnecessary for women who have node-negative disease. Sentinel nodes have been shown to be representative of the presence or absence of metastases in

the remainder of the nodal basin. Sentinel lymph node biopsy is increasingly being used to predict axillary node status in breast cancer on a world-wide basis. It allows directed therapeutic node dissections and confines the morbidity of the procedure to patients who will potentially benefit from removal of involved node.

Our approach to verify the hypothesis of sequential dissemination by dissecting the surgical specimen to identify sentinel nodes has several advantages over in vivo study. The specimen is easily accessible from all angles, so it is unlikely that blue nodes are missed. By tracing the lymphatic channels originating in the primary tumour, SNs can be distinguished from non-SNs. Not all blue-stained nodes are SNs as dye will sometimes spill over to non-SNs.

Additional benefits from our approach are that the duration of the operation is not extended and that the discomfort to the patients in the study is limited to blue-stained urine during the post-operative phase. The results of the current study show that patent blue dye injected in the primary tumour generally drains through the lymphatic channels. Although several intra-operative techniques including imprint cytology, frozen sectioning or a combination of these techniques have been examined. A key advantage of the imprint cytology over frozen sectioning is the avoidance of the loss of tissue attendant to the use of a cryostat. Thus, imprint cytology preserves tissue for subsequent focused pathologic analysis of the SLNs.

## Age

Previously studies have proved that patients undergoing SLN biopsy along with ALND are predominantly postmenopausal. (Langer et al, 2005)<sup>4</sup> From this study it is seen that postmenopausal women with a mean age of 63.5 had undergone SLN biopsy along with ALND as most of the IIC positive lymph nodes patients are coming under this group. In this study, we have seen that most of the patients having a comparatively large tumor size and SLN metastasis are coming under postmenopausal age group.

## Situation

The most common location of the tumour was the upper outer quadrant with great number of sentinel node detection as seen in many studies like Rubio et al in 1998.<sup>5</sup> In our study, 68.5% of our cases are having tumour in upper outer quadrant and out of which Sentinel lymph nodes were detected in 98% cases. Detection rate was same for lower outer and lower inner quadrants that is 83.5%, whereas central and upper inner quadrants have an identification rate of 50% only.

## Size of Mass

The mean diameter of the tumours was 2.9 cms. (range 1.1-5.0 cm). The prevalence of axillary metastases increases with increasing tumour size (Leidenius et al 2005, Viale et al 2005).<sup>3,6</sup> The prevalence of axillary metastases has been reported to be especially high among patients with multifocal tumours (Leidenius et al 2005, Andea et al).<sup>3,7</sup> Due to the higher prevalence of axillary metastases in connection with



large and multifocal tumours, there are evidently more numerous false-negative SNB cases associated with them, even though the false-negative rate is similar in small and large tumours.

**Histological Types of Invasive Breast Carcinoma**

The sensitivities of detecting pure invasive ductal carcinoma, invasive lobular carcinoma and other invasive carcinomas were 66.7%, 66.7%% and 55%, respectively. No statistically significant difference was observed in detection of pure invasive ductal and lobular carcinoma by intraoperative imprint cytology. One case each of medullary carcinoma and neuroendocrine carcinoma, coming under others are negative for SLN metastasis and these tumours are <3 cms. diameter was clinically suspected as fibro adenoma and fibrocystic disease respectively.

**Overall Accuracy**

Imprint smears have proved superior to frozen-sectioning due to the inherent disadvantages in the latter. Proponents of touch preparation claim that in the hands of an experienced cytopathologist, it may even be more accurate than permanent sections because it samples the entire surface area of the resected specimen.

Overall accuracy, sensitivity and specificity are 87.5%, 91% and 100% respectively.

Authors	Accuracy	Specificity	Sensitivity
Klimberg et al (1998) <sup>8</sup>	100%	100%	100%
Creager et al (2002) <sup>9</sup>	84%	80%	85%
Sauer (2003) <sup>10</sup>	94.3%	98%	-
Vinod Shidham (2004) <sup>11</sup>	-	100%	93.7%
Khanna AK (2010) <sup>12</sup>	-	100%	98.4%
Present Study	87.5%,	91%	100%

**Table 5**

**CONCLUSIONS**

Immediate and reliable intraoperative information on the status of the SLN would be beneficial for the surgeon to decide as to whether to do or not to do a complete axillary dissection at the time of the initial surgery. To summarize, SLN biopsy can identify a negative axilla with high accuracy. Women with negative SLNs may be spared axillary node dissection and its complications while receiving full staging information of the axilla. ALND could be performed only in patients with positive SLN or in those in whom a SLN cannot be found. As with other new techniques, there is a learning

curve associated with the procedure that all surgeons need to achieve.

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