

## ORIGINAL ARTICLE

## INTRA OPERATIVE FROZEN EXAMINATION OF SENTINEL LYMPH NODE IN BREAST CANCER

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**Background:** Sentinel node (SN) biopsy is the standard of care for the assessment of axilla in early breast cancer patients with clinically node negative disease. Confirmed absence of tumour deposit in node on intra operative frozen section (FS) examination saves the patient from complete axillary dissection. However controversies arise when inconsistencies occur in results of frozen and permanent section. Reported sensitivity of frozen examination of sentinel node in literature ranges from 70–95%. The purpose of this study was to determine the sensitivity of frozen examination of sentinel node in breast cancer. The frozen section examination of sentinel node is not a reliable technique for accurate pathological assessment of node. **Methods:** Data was collected prospectively on patients with sentinel node procedure from May to December 2013. All SNs removed at surgery were submitted for frozen section and the results were compared with permanent sections. **Results:** Of 50 patients 16 were true positive while 32 were true negative. Two patients reported negative on FS were confirmed to be positive on permanent section. The accuracy of frozen section was 96%, with sensitivity of 89%. In false negative cases the size of nodal metastasis was significantly smaller than that of true positive, i.e., 1–2 mm. The false negative cases were further classified for assessment into technical and interpretative error. **Conclusion:** The intra operative frozen section examination is a reliable technique for the assessment of Sentinel node with a high accuracy rate to detect metastasis size of  $\geq 2$ mm. It spares the patient from complete axillary dissection and its subsequent morbidity of lymphedema and shoulder pain.

**Keywords:** Sentinel node, frozen section, Breast cancer.

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## INTRODUCTION

Axillary nodal status has traditionally been the most significant factor in staging breast cancer,<sup>1</sup> it is an independent predictor of survival and risk of recurrence in breast cancer. Although; axillary lymph node dissection (ALND) has been a standard of surgery for breast cancer, however the procedure has associated morbidity of impaired shoulder function, lymphedema and paresthesia. To reduce the morbidity of axillary dissection the concept of sentinel lymph node (SLN) evolved for patients with early breast cancer. the SLN are sensitive and specific predictors of status of non SLN s in breast cancer.<sup>1,2</sup> NSABP-32 TRIAL looking at SLN negative group showed overall survival, disease free survival and regional control were statistically equivalent between two groups, i.e., those who underwent SLN with axillary dissection or SLN surgery alone.<sup>3</sup> The results from Z0011 trial looking in the context whether additional axillary dissection in limited SLN metastatic breast cancer affects the survival showed that women with T1-T2 tumour with positive SLN undergoing lumpectomy with radiation and systemic treatment do not benefit from additional ALND in terms of local control and overall survival.<sup>4</sup> So SLN is a safe and effective therapy for patients with breast cancer with clinically negative lymph nodes.

However; controversies exist with respect to intra-operative assessment of SLN. It is easier to miss micro metastasis with a conventional Frozen section (FS) technique in SLN than with permanent section.<sup>5</sup> Intra operative evaluation of these nodes should be performed accurately as a false negative result would entail a second surgical procedure to complete staging with incurred cost and additional morbidity of second surgery and negative psychological impact on patient.<sup>4</sup>

According to 7<sup>th</sup> AJCC cancer staging manual Isolated tumour cell clusters (ITC) are defined as small clusters of cells not greater than 0.2 mm, or single tumour cells, or a cluster of fewer than 200 cells in a single histologic cross-section & micro metastases are defined as tumour deposits greater than 0.2 mm but not greater than 2.0 mm in largest dimension. Cases in which only micro metastases are detected (i.e., none greater than 2 mm) are classified pN1mi.

In this study we describe our experience of routine intra operative FS examination of SLN in women with early breast cancer, we attempted to identify false negative rate, sensitivity, accuracy of the procedure and factors contributing to false negative results. The study was approved from IRB Committee of Dow University of Health Sciences.

**MATERIAL AND METHODS**

The data was collected prospectively of 50 consecutive patients admitted in Dow university hospital from May to December 2013. All patients were diagnosed with early invasive breast cancer (T1-T3) with clinically impalpable lymph nodes and additional sonographic evidence of benign lymph nodes. Blue dye was injected periareolar in sub dermal plane for localization & identification of sentinel node. SLN was successfully visualized and all blue nodes in addition to those found suspicious pre-operatively on palpation were subjected to FS examination and permanent H&E staining. Each node was analyzed by a pathologist for gross and microscopic evaluation. Nodes <1 cm in size were oriented along longitudinal axis and bisected in two, while nodes >1cm were serially sectioned. Sections were submitted for frozen section using H&E staining. The process was performed by trained technologists, under supervision of specialist pathologist. All slides were read by consultant pathologist and diagnosis conveyed to surgeon in operating room. Axillary dissection/clearance was added for patients with positive reports. At the final analysis all SLN were submitted for permanent section with H&E staining. Results of intra-operative FS assessment of the SLN were then compared with permanent section. Data was entered on SPSS version 19 and sensitivity, accuracy, negative predictive value and false negative rate were calculated from 2x2 tables.

**RESULTS**

A total 50 female patients were included in the study from May to December 2013. Age of patients ranged from 28–75 years, with mean of 55years. Sentinel node was successfully localized in all patients. Total 198 SLN nodes were removed (maximum 4 and minimum 1). Tumour size was T1 in 2, T2 in 44, and T3 in 4 patients. Infiltrating ductal carcinoma diagnosed in 46 patients, lobular in 3, and mucinous in 1 case. Using Allred scoring system 40 were ER/PR positive, while 8 were ER/PR negative & 2 were ER positive PR negative. (Table-1)

Out of 50 patient 16 were true positive, 32 were true negative, 2 were false negative and none of them were false positive. (Table-2) The sensitivity and specificity were 89% and 100% respectively, while positive predictive value and negative predictive value

were found to be 100% and 94.1%. Accuracy and false negative rate were 96% & 11% respectively. (Table-2)

Additional surgery on axilla with false negative report was not undertaken as the number of removed nodes (4) in both patients were adequate for staging. Size of tumour deposit was 1–2 mm, rendering it clinically negative according to AJCC classification. (Table-3)

To delineate errors in false negative case, two senior pathologists reviewed frozen slides. Technical error was identified in both cases, disruption of capsule during handling rendered missing of a sub capsular deposit.

**Table-1: Patient Characteristic**

<b>Total number of cases</b>	<b>50</b>
Age in years. (range)	55 (28–75)
Slnb Method(Blue Dye)	50
<b>Tumour size</b>	
T1	2
T2	44
T3	4
Total	50
<b>Histology</b>	
Invasive Ductal	46
Invasive Lobular	3
Mucinous	1
Total	50
<b>Receptor status</b>	
ER/PR+VE	40
ER/PR-VE	8
ER+/PR-	2
<b>GRADE</b>	
Grade-I	2
Grade-II	46
Grade-III	2

**Table-2: Predictive value of frozen section of axillary status**

	<b>Histopathology(permanent section)</b>	
<b>Frozen section</b>	+	-
+	True positive 16	False positive 00
-	False negative 2	True negative 32
<b>Total</b>		

Sensitivity 89 %,specificity 100% , positive predictive value 100%, negative predictive value 94.1 %, accuracy 96%, false negative rate 11%.

**Table-3: Metastatic size in SLN**

<b>SLN frozen section result</b>	<b>N</b>	<b>Metastatis size (mm)in SLN (mean±SD)</b>
False negative	2	1.5 mm (1–2)
True positive	16	3 mm (1–25)

**Table-4: Literature Review**

Reference	Year	Number of patients	Intraoperative technique Stains no. of level	Final technique Stains no. of level	FNR Percentage	Accuracy Percentage
Veronesi <i>et al</i>	2001	295	H&E multiple	-	2.7	
Motomura <i>et al</i>	2000	101	H&E single	H&E multiple	3.2	88.1
Vrande <i>et al</i>	2009	615	H&E multiple	H&E multiple IHC	7	90.7
Sharma <i>et al</i>	2009	40	H&E multiple	H&E multiple IHC	4.2	97.5
Schwartz <i>et al</i>	2010	283	H&E multiple	H&E multiple	4.7	97
Rohana <i>et al</i>	2008	94	H&E multiple	H&E multiple IHC	9.85	92.55
Arlicot C <i>et al</i>	2013	672	H&E multiple	H&E multiple	40	--
Wada <i>et al</i>	2004	569	H&E single	H&E single	16	97
DUHS	2013	50	H&E multiple	H&E multiple	11	96

## DISCUSSION

With an increase in awareness and screening methods, early breast cancer is on a rise. With a high probability of pathologically negative nodes, the axillary dissection may constitute an excessive treatment knowing the fact that preservation of healthy lymph nodes has beneficial consequences. To date, none of the available imaging modalities are sensitive enough to replace pathological confirmation in early breast cancer patients. Sentinel node biopsy is a reliable procedure to determine presence of nodal disease prior to therapeutic axillary surgery.<sup>1-3</sup>

Sentinel lymph node (SLN) biopsy, introduced about 15 years ago, with a high identification rate and false negative rate ranging from (4.7–16.7%), and reported accuracy of 95–100% has now become the standard in management of clinically node-negative breast cancer patient.<sup>6-8</sup> The most favourable combination of predictive factors may have no less than 13–60% risk for non-sentinel lymph node metastases requiring ALND.<sup>6-10</sup>

Following primary surgery, conventional histological evaluation of patients in the event of positive node would incur a second operation on axilla. This could be avoided with accurate intra operative histological evaluation of sentinel node. Accurate intra operative assessment therefore, is the mainstay, of extending a diagnostic procedure to identify therapeutic needs.<sup>5,11</sup>

The importance of size of tumour deposit in SLN cannot be overlooked. Size of metastatic deposit is determinant of absolute detection on Frozen Section as well clinical significance, translating into treatment strategies. Micrometastasis or ITC detected on FS axillary node dissection can be easily omitted as reported literature shows that micrometastasis or ITC does not translate into positive axilla as there is no significant difference in loco regional, axillary and distant disease-free survival between patients with negative SLN and SLN micrometastasis.<sup>12,13</sup>

A multiple of techniques have been used for correct identification of metastatic deposit in SLN per operatively. The techniques in use for intra operative assessment of SLN includes touch imprint cytology, frozen section and rapid immune histochemistry. The two common methods are frozen sections and imprint cytology.<sup>14</sup> In literature sensitivity of imprint cytology varies between 36.5–96% and specificity from 90.8 to 100%.<sup>14,15</sup> This has been attributed to poor quality of the imprints, sub capsular tumours cell deposits, lack of imprinting of large cohesive groups of epithelial cells. Size of metastasis has also been addressed with small size are more likely to be missed, along with low grade tumours, lobular

carcinomas and diffuse unicellular infiltrating pattern may remain undetected.<sup>15,16</sup>

The frozen section analysis is considered to be a reliable, safe and cost effective method for intra-operative examination of SLN. It requires experienced technical staff and it is time consuming. Additional limitation is sampling error, freezing artifacts and 20–25% tissue loss during processing.<sup>15</sup> Technical and interpretative error largely accounts for false negativity. Low sensitivity reported in many studies largely accounts because of single section examination which can easily missed micro metastasis. Additional multiple section examination may identify as many as 16.7% cases.<sup>5</sup> Reported sensitivities of frozen section are from 52–91%, accuracy of FS rests between 79–98%, and a false-negative rate of 4–45%.<sup>17-27</sup> (Table-4)

Veronesi *et al* emphasize that with a change in their conventional intra operative FS method to extensive examination of SLN almost 60 sections per node, increased their accuracy from 92–95%. While they showed their method to be highly sensitive, this could be interpreted as overstating in the face of uncertainty of clinical significance of micro metastasis.<sup>5</sup>

Motomura *et al* report low sensitivity of 52.2% with only one section taken for frozen section.<sup>17</sup> Sharma *et al* reported that serial sectioning increases the rate of detection of micrometastasis and chances of equivocal results are lower than imprint cytology with sensitivity of 95.8%.<sup>18</sup> We perform an accurate search for micrometastasis and for ITC during the definitive histopathological examination, also by performing the immune histochemical assay for cytokeratins in all the cases of negative and ambiguous haematoxylin–eosin results.

The role of IHC in the setting of micrometastasis has been observed to be more sensitive than imprint cytology and frozen section. When added to serial sectioning, results of IHC are equivalent to paraffin sections.<sup>15</sup>

We used conventional method for frozen section, i.e., technique of careful isolation of SLN from the surrounding fat tissue by surgeon without breaking the node capsule. This was followed by processing of node by senior technician and histo pathologist who includes multiple serial section of node with correct orientation readily reflects in our results, i.e., accuracy of 96%. The published results from Veronesi *et al* and Schwartz *et al* studies confirmed the fact that rough handling of node and single section increase the false negative rate.<sup>5,18</sup>

To analyze our false negative case senior pathologist reviewed all the frozen slides to delineate the error. Technical error was detected as Capsule

disruption during tissue handling contributed to non-identification of sub capsular deposit in both cases.

Previous published data also correlated sensitivity of FS in regard to histological type, tumour size, lympho vascular invasion and size of tumour deposit. There is a positive correlation between size of tumour deposit and FS sensitivity as in both false negative cases deposit size was 1–2 mm. Wada *et al*<sup>20</sup> also validated that micrometastasis can easily be missed in frozen analysis, Vrande *et al*<sup>21</sup> reported sensitivity of FS was significantly greater when contained macrometastasis vs micrometastasis (84% vs 61%), Calogero *et al*<sup>22</sup> reported 90% vs 12.5%. Krishna murti *et al*<sup>23</sup> reported sensitivity 100% vs 75% respectively.

Histological type, tumour size and lympho vascular invasion (LVI) had no significant impact on frozen sensitivity in our study. In both of cases tumour type was ductal carcinoma, having T2 size & none of them showed LVI. This is contrary to findings reported by Chan *et al*<sup>24</sup> that yield of frozen is more with bigger tumour size, positive LVI and in IDC in comparison to ILC, i.e.; 62% vs. 52%. This was not observed by Calogero *et al* and Horvathetal.<sup>22,25</sup>

The possible explanation of our findings was the fact that in both cases sub capsular metastatic deposits were missed because of technical error.

## CONCLUSION

Intraoperative frozen examination of SLN is a useful predictor of axillary status which allows the surgeon to extend SLNB to complete axillary dissection in the event of positive SLN. Accuracy and sensitivity of the procedure however is linked with technique and size of tumour deposits.

Needless to say, preoperative counselling of patients with respect to false positive and negative rates pertaining to the surgeon's validation figures cannot be overemphasized.

## CONFLICTS OF INTEREST

There are no conflicts of interest of any authors.

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