

## RESEARCH ARTICLE

# Accuracy of Frozen Section Analysis of Sentinel Lymph Nodes for the Detection of Asian Breast Cancer Micrometastasis - Experience from Pakistan

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### Abstract

**Background:** Intraoperative sentinel lymph node biopsy has now become the standard of care for patients with clinically node negative breast cancer for diagnosis and also in order to determine the need for immediate axillary clearance. Several large scale studies confirmed the diagnostic reliability of this method. However, micrometastases are frequently missed on frozen sections. Recent studies showed that both disease free interval and overall survival are significantly affected by the presence of micrometastatic disease. The aim of this study was to determine the sensitivity and specificity of intraoperative frozen section analysis of sentinel lymph nodes (SLNs) for the detection of breast cancer micrometastasis and to evaluate the status of non-sentinel lymph nodes (non-SLNs) in those patients subjected to further axillary sampling. **Materials and Methods:** We performed a retrospective study on 154 patients who underwent SLN biopsy from January 2008 till October 2011. The SLNs were sectioned at 2 mm intervals and submitted entirely for frozen sections. Three levels of each section submitted are examined and the results were compared with further levels on paraffin sections. **Results:** Overall 40% of patients (62/154) were found to be SLN positive on final (paraffin section) histology, out of which 44 demonstrated macrometastases (>2mm) and 18 micrometastases (<2mm). The overall sensitivity and specificity of frozen section analysis of SLN for the detection of macrometastasis was found to be 100% while those for micrometastasis were 33.3% and 100%, respectively. Moreover 20% of patients who had micrometastases in SLN had positive non-SLNs on final histology. **Conclusions:** Frozen section analysis of SLNs lacks sufficient accuracy to rule out micrometastasis by current protocols. Therefore these need to be revised in order to pick up micrometastasis which appears to have clinical significance. We suggest that this can be achieved by examining more step sections of blocks.

**Keywords:** Micrometastasis - sentinel lymph node biopsy - breast cancer - frozen section

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### Introduction

Breast cancer has proffered as a serious public health dilemma for many years and has romped havoc with life of many patients. It is the leading cause of cancer morbidity and mortality among women all over the world. The reported incidence of reproductive age breast cancer is highest globally (Bhurgri et al., 2007). In Karachi, it annals for one thirds of female malignancies. This actuality has established major concerns regarding the clinical management of breast cancers. Axillary lymph node metastasis is the most important prognostic factor in breast cancer and therefore has major clinical implication on its management (Fisher et al., 1983).

Sentinel lymph node (SLN) biopsy became standard of care for clinically node negative breast cancer patients

because it has significantly reduced the morbidity of breast surgery (Giuliano et al., 1994; Veronesi et al., 1997). This technique involves the identification of first node(s) draining the tumor area by the introduction of a vital blue dye or radio-labelled substance. SLNs are examined by frozen section or imprint cytology and if they are positive for malignancy, axillary lymph node dissection (ALND) is carried out in same period of time. On the other hand if they are negative for tumor, the probability of tumor metastasis in non-sentinel lymph nodes (non-SLN) is very low (Veronesi et al., 1999; Viale et al., 1999), so surgeons can relinquish the ALND procedure. However limited axillary sampling can be done if the index of suspicion is high.

The American Joint Committee on Cancer (Edge et al., 2010) classified tumor deposits of less than 2 mm in

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diameter in SLNs into micrometastasis and isolated tumor cells (ITCs). Micrometastasis is defined as metastasis that are larger than 0.2 mm in diameter but 2 mm or smaller, denoted as lymph node positive (pN1mi), whereas Isolated tumor cells are defined as tumor cell clusters that are more than 0.2 mm in largest diameter and are denoted as lymph node negative [pN0(i+)]. Tumor deposits which measure more than 2 mm are referred as macrometastasis.

Although SLN biopsy has contributed to conspicuous decrease in the overall morbidity of breast surgery, however a consensus has not been reached regarding its standardization. This is primarily because the clinical significance of previously unrecognized micrometastasis is still unclear. The sensitivity of frozen section of SLNs in the detection of micrometastasis also varies from institution to institution because it largely depends on the method of analysis and the extent of sampling adopted. The aim of this study is to determine the accuracy of intraoperative frozen section analysis of SLNs for the detection of breast cancer micrometastasis and to evaluate the status of non-SLNs in those patients who are subjected to further axillary sampling.

**Materials and Methods**

We performed a retrospective observational study on 154 patients who underwent breast surgery for clinically node negative breast cancer from January 2008 till October 2011. An approval from institutional ethical review committee was obtained antecedent to conducting the study. Informed consent from patients was obtained prior to surgery. To perform SLN biopsy procedure, lymphoscintigraphy was done. In this procedure <sup>99m</sup>Tc-albumin nanocolloid was prepared and then injected in the subareolar area of the patient. Scintigraphic imaging was acquired using gamma camera within 5 minutes after the injection. During the biopsy a gamma camera was used to determine which nodes had absorbed the radionuclide dye. These lymph nodes which may be one to several in number were labelled as SLNs; they were dissected and sent to histopathology laboratory for frozen section analysis. The received SLNs were sectioned at an interval of 2 mm and submitted entirely for frozen section. The 6-10 um thick sections were made at three levels from each piece submitted and stained with hematoxylin and eosin (H&E) stains. The frozen section results were reported as negative or positive for metastasis. In all those cases where the result of frozen section is positive for metastasis (either macro or micrometastasis) complete ALND was carried out at the same time. On the other hand when the result of frozen section was negative, patients were subjected to no further axillary sampling. After the frozen section results were reported, all pieces were paraffin embedded and one to three further levels were examined. One case of micrometastasis was also confirmed by cytokeratin (AE1/AE3) immunohistochemical stain. The results of frozen and paraffin sections were both mentioned in the final report. Furthermore histological type of tumor, extent of invasion, tumor grade and non-sentinel axillary lymph node status were also recorded.

**Results**

Out of 154 patients who were included in the study, 34% of patients had modified radical mastectomy, while the rest underwent breast conservation surgeries including, lumpectomy with or without axillary dissection and simple mastectomy (Figure 1). The breast cancer incidence was most common age group between 30-50 years (Figure 2). This also coincides with the national data as discussed previously.

Most of the tumors were in stage T2 range (Figure 3). Infiltrating ductal carcinoma comprised the most common subtype of the tumor accounting for 80% of the cases (Table 1) and tumors were grade II (Figure 4). The number of SLNs per patient ranged from 1 to 5 with a mean of 2.3. Overall 40.3% of cases (62/154) of SLNs were positive

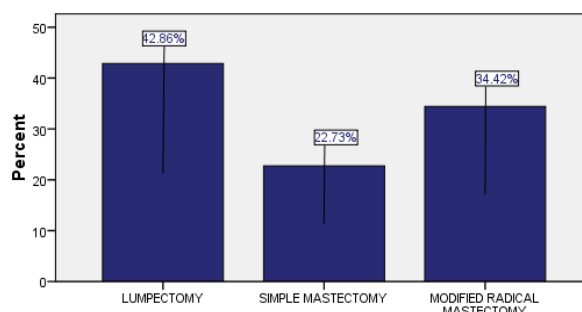


Figure 1. Type of Surgical Specimens

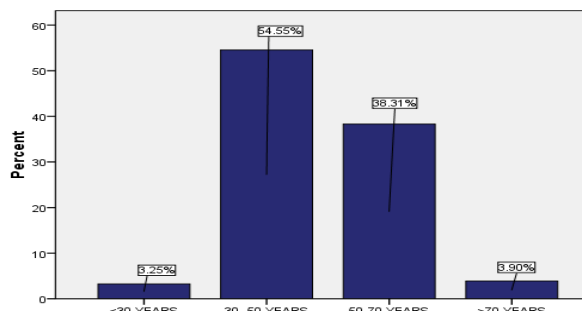


Figure 2. Age Distribution

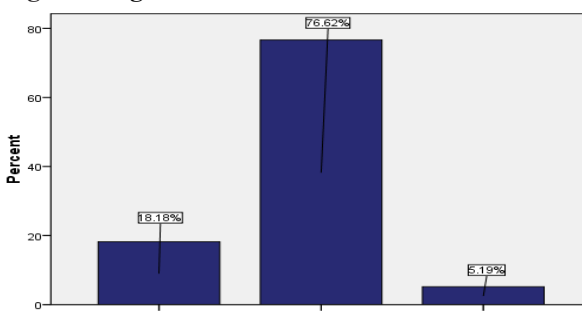


Figure 3. Tumor Size Distribution

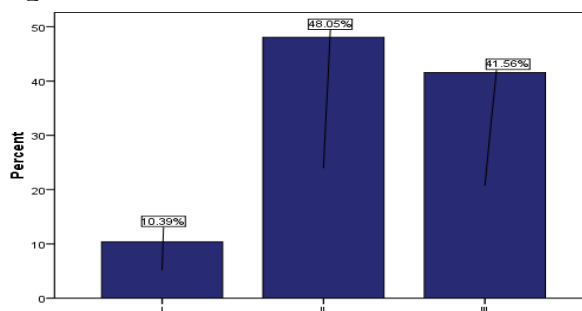


Figure 4. Tumor Grades

on final histology out of which 44 were macrometastasis and 18 were micrometastasis.

Table 2 compares the results of frozen sections and corresponding results of paraffin sections. A total of 154 cases of SLNs were examined, out of which 50 were positive on frozen section. There were 12 cases in which frozen section results were discordant with the final paraffin section diagnosis and all these cases were that of micrometastasis.

Table 3 further emphasizes the results of SLN biopsy with breakdown of positive results into macrometastasis and micrometastasis. It shows that all 44 cases of macrometastasis were correctly identified on frozen section, on the other hand in cases of micrometastasis 6 out of 18 cases were correctly diagnosed on frozen section and 12 cases which were labelled negative at time of frozen section, the diagnosis was changed with paraffin section results giving a false negative rate of 66.7%.

Table 3 summarizes the overall sensitivity and specificity of frozen section analysis of SLNs. The overall sensitivity is 80.6% with 100% specificity. For

**Table 1. Types of Tumor**

	Tumor type	n	%
Infiltrating ductal carcinoma		124	80.5
Infiltrating lobular carcinoma		2	1.3
Medullary carcinoma		2	1.3
Mucinous carcinoma		1	0.6
Tubular carcinoma		3	1.9
Metaplastic carcinoma		4	2.6
Tubulolobular carcinoma		2	1.3
Infiltrating carcinoma(cannot be specified)		12	7.8
Total		154	100

**Table 2. Sentinel Lymph Nodes, Frozen Section Diagnosis vs. Paraffin Section Diagnosis**

		Paraffin section diagnosis		Total
		Positive	Negative	
Frozen section diagnosis	Positive	n	50	50
		Frequency	80.60%	
Negative	n	12	92	104
Total	n	62	92	154

**Table 3. Sentinel Lymph Nodes, Frozen Section Diagnosis vs. Paraffin Section Diagnosis with Comparison of Macrometastasis and Micrometastasis**

			Paraffin		Total
			Positive	Negative	
Macrometastasis	Frozen Section	Positive	44		44
Micrometastasis	Frozen Section	Positive	6		6
		Negative	12		12
	Total		18		18
Negative Sentinel Lymph Nodes	Frozen Section	Negative		92	92
Total	Frozen Section	Positive	50	0	50
		Negative	12	92	104
Total			62	92	154

**Table 4. Sensitivity and Specificity of Frozen Section Biopsy of Sentinel Lymph Nodes**

Variable	Sensitivity	Specificity
Frozen section of sentinel lymph node biopsy	80.60%	100%
Macrometastasis	100%	100%
Micrometastasis	33.30%	100%

**Table 5. Sentinel Lymph Nodes vs. Axillary (non-sentinel) Lymph Nodes**

	Axillary (non-sentinel) lymph nodes		Total n
	Positive	Negative	
Sentinel Lymph Nodes			
Macrometastasis	19 (43.2%)	25 (56.8%)	44
Micrometastasis	2 (20.0%)	8 (80.0%)	10
Negative S.L.Nodes	5 (9.3%)	49 (90.7%)	54
Total	26 (24.1%)	82 (75.9%)	108

**Table 6. Sentinel Lymph Nodes vs. Lymphovascular Invasion**

	Lymphovascular invasion		Total
	Present	Not Present	
Sentinel Lymph Nodes			
Macrometastasis	13 (29.5%)	31 (70.5%)	44
Micrometastasis	2 (20.0%)	8 (80.0%)	10
Negative S.L.Nodes	4 (7.4%)	50 (92.6%)	54
Total	19 (17.6%)	89 (82.4%)	108

macrometastasis detection the sensitivity was 100%, however in the cases of micrometastasis the sensitivity is only 33.3% with 100% specificity.

Out of total 154 patients, 108 patients underwent further axillary dissection after SLN biopsy. Table 4 shows the frequency of non-SLN metastasis in all these patients. All 44 patients who had positive SLN with macrometastasis had complete axillary dissection and the nodes thus recovered were designated as non-SLNs. Out of these 44 cases of macrometastasis, 19 (43.2%) cases had positive non-SLNs. Out of total 18 patients who had a final diagnosis of micrometastasis in SLNs, 10 had further axillary dissection/sampling, out of which 2 (20%) had positive non-SLNs. Finally out of 92 patients who had negative SLNs, 54 patients underwent further axillary sampling, out of which 5 (9.3%) were positive for metastasis.

Table 6 shows the frequency of lymphovascular invasion which is considered as a marker of nodal metastasis in these patients. In those patients who had positive SLNs with macrometastasis and micrometastasis, 13 (29.5%) and 2 (20%) cases had lymphovascular invasion respectively. Even in patients with negative SLNs 19 (17.6%) cases were positive for lymphovascular invasion.

## Discussion

Breast cancer is the most frequent cancer of women in Karachi, accounting for one third of cancers in women. The tumor burden of breast cancer in our population is highest in Asia (Bhurgri et al., 2006).

SLN biopsy is an area of extensive research in the

staging and management of breast cancer. By reducing the number of nodes to be examined, more exhaustive histopathological approach for examining SLNs become possible. Intraoperative analysis of SLNs can be done by imprint cytology or frozen sections. Frozen section evaluation is slightly superior to imprint cytology. Numerous studies evaluated the accuracy of intraoperative frozen section and the sensitivity depends on the method of examination adopted. A meta-analysis reported the sensitivity of intraoperative frozen section, ranging from 57-74% (Layfield et al., 2011). The detection rate for micrometastasis in other studies is reported to range from 9-46% (Dowlatshahi et al., 1999; Tille et al., 2009), whereas in our study the overall sensitivity was 80.6% and for micrometastasis the sensitivity was found to be 33.3%.

SLN is a very labor intensive technique but still controversies exist around its protocol. This is primarily because current data from large scale clinical trials is still insufficient to answer two central questions. First, what is the frequency of non-SLN metastasis in the absence of SLN involvement and long term axillary recurrence, and second, the clinical significance of micrometastasis and ITCs which are frequently missed on frozen sections. Regarding first, multiple studies evaluated this issue and reported the frequency of non-SLN metastasis ranging from 5-13% (Giuliano et al., 1994; Krag et al., 1998; Turner et al., 1999; Weaver et al., 2000) with negative SLNs. O'Hea et al. (1998) in a study noted involvement of non-SLNs in 5 patients with a frequency of 13%. Giuliano et al. (1994) similarly found non-SLN metastasis in 11% of cases in a study of 174 patients. In our study 5 out of 54 patients who had negative SLNs demonstrated non-SLN metastasis with a frequency of 9.3%.

The most difficult question to answer which posed frequent problems for breast surgeons, pathologists and researchers is to determine the clinical significance of micrometastasis. This is because most of the studies to date are hampered due to lack of long term follow up and short sample size in order to clearly define the clinical relevance of micrometastasis and isolated tumor cells. Cohort studies reported the survival rates in patients with micrometastasis or isolated tumor cells and found no reduced recurrence free survival (Liang et al., 2001; Chagpar et al., 2005; Fan et al., 2005; Soni et al., 2005; Imoto, 2006; Nagashima et al., 2006). On the other hand two studies reported a higher recurrence rate for patients with micrometastasis (Rydén et al., 2007; Cox et al., 2008) They also found additional metastasis in non-SLNs.

It is well established that the need for complete ALND in T1 and T2, clinically node negative breast cancer depends on the results of sentinel lymph node biopsy, however the indications are evolving (Sanuki et al., 2013). Although there is no question on the performance of complete ALND if micrometastasis or macrometastasis are found in three or more nodes, however the need for a complete ALND in patients with a positive SLN showing micrometastases or macrometastases in less than three nodes has been argued (Carlson et al., 2011). The SLN is the sole tumor-bearing node in up to 60% of cases overall, and in almost 90% of patients harbor only micrometastatic disease. These observations have led to speculation that

complete ALND may not be necessary in selected patients with a positive SLN in less than three nodes because the need for systemic therapy is established and the risk of an axillary recurrence appears to be low.

The American College of Surgeons Oncology Group (ACOSOG Z-0011) trial was designed to address the need for complete ALND for patients with T1 or T2 tumors that were clinically node negative and had less than three positive SLNs (Giuliano et al., 2010; 2011); all patients were treated with radiation to the breast. The five-year overall survival was similar whether women were treated with SLND plus ALND or with SLN biopsy alone (91.9 versus 92.5 percent, respectively). Recurrence rates in the ipsilateral axilla were similar between the two arms with four recurrences (0.9%) in the SLN biopsy alone arm compared with two recurrences (0.5%) in the ALND arm.

The International Breast Cancer Study Group trial 23-01 (IBCSG 23-01) randomized patients with SLN micrometastases (<2 mm) and primary tumors <5 cm in size to either completion ALND or SLN biopsy alone (Galimberti V; International Breast Cancer Study Group Trial 23-01). The study included 931 patients. With a median follow-up of 49 months, there was no significant difference in disease free survival rate for patients treated with an ALND compared with those treated with a SLN biopsy alone (87 versus 92%). There was no significant difference in overall survival rate for patients treated with an ALND compared with those treated with a SLN biopsy (97.6 versus 98.0%).

In 2013, we are still debating and trying to understand the clinical significance of micrometastasis in breast SLNs. In an analysis of population-based data from the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) national cancer database showed that the presence of micrometastasis in lymph nodes is associated with an overall decrease in survival at 10 years of 1% for T1, 6% for T2, and 2% for T3 breast cancers when compared to patients with negative axillary nodes (Chen et al., 2007). As new more and more studies are coming up regarding prognostic value of micrometastasis, our study also showed non-SLN involvement in 2 cases (20%) with micrometastatic disease in SLN and 5 cases (9.3%) with negative SLNs. Therefore we suggest that more intensified pathological assessment of SLNs need to be done.

In our study we have not performed molecular studies on SLNs to detect micrometastasis/ITCs due to unavailability of this facility at our setup. Considering the existing controversy about significance of micrometastasis, reverse transcriptase – polymerase chain reaction (RT – PCR) which having been studied for several years is still not recommended for detecting micrometastasis/ITCs in axillary lymph nodes. Moreover in our study, immunohistochemical stains were not performed on SLNs in every case, which may be the reason why ITCs were not detected in any case.

There is no standard agreement regarding how to examine SLNs and institutions have developed their own in-house protocols to evaluate SLNs. AJCC does not specify a grossing protocol for SLN biopsy. According to College of American Pathologists and American Society

of Clinical Oncology guidelines, SLNs should be sliced at an interval of 2 mm and one level should be examined from each block. This protocol is particularly designed for the detection of macrometastasis. However they stated that, the detection of micrometastasis can be enhanced by examining more step sections of the block.

Although large scale clinical trials to determine the clinical significance of occult metastasis are underway but it seems likely from the results of a few recent studies that micrometastasis do appear to have clinical significance and impact on long term survival. Detection of micrometastasis in SLN is a grave challenge but hypothetically speaking its detection can be enhanced by examining whole SLN at predetermined intervals.

In conclusion, frozen section analysis of SLNs lacks sufficient accuracy to rule out micrometastasis by current protocols which include examining sections at an interval of 2 mm. Presence of micrometastasis in SLNs appears to increase the likelihood of positive non-SLNs and perhaps the risk of axillary recurrence and long term disease free survival. Examining more step sections or perhaps whole node at evenly spaced levels (200- to 500-um) can increase the sensitivity of frozen section for the detection of micrometastasis. However more large scale prospective studies are needed to prove the clinical significance of micrometastasis, so that a standard protocol can be devised for SLN evaluation.

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