Original Research Article

Is there a role of frozen section of sentinel lymph node in patients with early breast cancer for the management of axilla in India: a prospective study

Anshika Arora[1]*, Neena Chauhan[2], Sunil Saini[1], Nishish Vishwakarma[1], Tanvi Luthra[1]

[1]Department of Surgery, [2]Department of Pathology, Cancer Research Institute, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun, Uttarakhand, India

*Correspondence:
Dr. Anshika Arora,
E-mail: anshika00mittal@gmail.com

ABSTRACT

Background: Evaluation of axilla using sentinel lymph node biopsy (SLNB) is the standard of care in node negative early breast cancer. Intra operative assessment of SLNB with frozen section (FS) often guides the surgeon regarding decision for level of axillary dissection. The aim of this study was to evaluate accuracy of FS of SLNB in these patients with histopathology examination (HPE) as the gold standard.

Methods: This study was performed between July 2017 and November 2018. After gross evaluation of SLNB, nodes were cut in half and frozen; the other half was preserved for HPE. For FS, nodes were sectioned to 4 mm width and examined.

Results: A total of 61 patients underwent SLNB, 55 patients undergoing intra-operative FS. The mean age was 53 years (range 30-84, ± 15.09 SD), primary tumor was clinically T1 in 23.6%, T2 in 76.4% patients. A median of four sentinel nodes were identified, mean size 13.84 mm. On FS SLNB was positive for metastasis in 14 (25.5%), on HPE in 16 (29.1%) patients. There were 13 true positive, 38 true negative, 3 false negative and 1 false positive result for FS. The sensitivity, specificity, positive and negative predictive value, false negative and false positive rates were 81.25%, 97.44%, 92.86%, 92.73%, 18.75% and 2.56% respectively in this study. The overall accuracy of FS of SLNB in early carcinoma breast was found to be 92.73%.

Conclusions: An intra-operative FS of the SLN in node negative early breast cancer is a highly sensitive tool in axilla management.

Keywords: Axilla management, Early breast cancer, Frozen section, Negative predictive value, Positive predictive value, Sentinel lymph node biopsy, Sensitivity, Specificity

INTRODUCTION

Worldwide, breast carcinoma is the commonest cancer of women, with approximately 1.67 million new cases diagnosed in the year 2012, making up for 25% of all female cancers. Among the most common causes of cancer-related mortality worldwide, it ranks fifth, but is the most common cause of cancer related mortality in less developed countries.[1] With current treatment regimens, almost 90% of breast cancer patients may survive up to 5 years. This survival is found strongly associated with the stage of disease at the time of diagnosis.[2] The management of breast cancer can be broadly divided into three categories- early breast cancer, locally advanced breast cancer and metastatic breast cancer. According to NCCN guidelines patients with early breast cancer with TNM staging T0, N1, M0 and TI-3, N0-1, M0 should be managed according to the

clinical node negative or positive disease status. Clinical node negative is defined as non-palpable nodes and mammographically negative nodes in the axilla. Any patient with clinical node positive disease should be evaluated with a fine needle aspiration cytology (FNAC) or core needle biopsy of the node. FNAC or core biopsy negative nodes are considered clinically node negative. All patients with above TNM stage and clinical node negative axilla should undergo an axillary staging with sentinel lymph node biopsy (SLNB).[3] In early stage breast cancer the status of the axillary lymph nodes is a strong prognostic factor and SLNB is now the standard staging procedure to assess metastasis to the axillary lymph nodes.[4] In contrast, axillary lymph node dissection (ALND) was conventionally, a regular element in surgical management of even early breast cancer. The advantages of ALND included- benefit on disease control like recurrence in axilla and thus survival, as a prognostic procedure, and it thus helped in adjuvant treatment selection. On the down side, the lymphatic disruption caused by ALND may give rise to lymphedema, shoulder dysfunction and chronic pain due to varying degrees of nerve injury, these eventually affect the functional outcome, as well as the patient's quality of life negatively. An intra-operative evaluation of SLNB with good accuracy can help the surgeon with decision for level of axillary dissection (AD) and spare the morbidity of complete level III ALND or second surgical procedure.[5] Although a histopathology of lymph nodes using a standard hematoxylin and eosin staining (HPE) is recommended by the American Society of Clinical Oncology guidelines, surgeons frequently request intra-operative assessment of SLNB using frozen section (FS) technique.[6,7] There are some potential problems with the FS method-loss of fatty nodal tissue, interpretation of artifact impacting and lack of standardized method for FS evaluation of SLNB. This may result in extensive inter-observer variability due to the quantity of nodal tissue examined and also due to the number of sections examined for every specimen.[8] This prospective study was designed to study the sensitivity and specificity of FS of SLNB with HPE as gold standard.

METHODS

This prospective observational study was performed at Cancer Research Institute, Swami Rama Himalayan University, Dehradun, India between July 2017 and November 2018, after an institutional ethics clearance. All carcinoma breast patients with TNM stage TI-3, N0, M0 with clinically node negative axilla were evaluated for enrolment in the study, after a written informed consent. Clinical node negative axilla was defined as - no palpable nodes and mammographically negative nodes.

Exclusion criteria

- FNAC or core biopsy proven axillary node metastasis in clinically N1 axilla.
- Previous ipsilateral axillary surgery.

The demographic and disease related data was collected for the patients. At the time of definitive surgery for the primary tumor all patients underwent SLNB. The SLNB tissue was submitted for intra-operative FS. A gross evaluation was performed first to establish the number of nodes and grossly suspicious nodes. All nodes were measured for size, nodes up to 4 mm were frozen whole, rest were bisected into half-one for FS and one preserved for HPE. For FS, the nodes were sectioned to 4 mm width and examined. Definition of nodal macrometastasis was - tumor nest more than 2 mm in diameter; micrometastasis was- tumor nest greater than 0.2 mm and less than or equal to 2 mm. Presence of extra-nodal involvement by tumor was also noted if metastases were detected. Data was collected for presence of nodal metastasis on FS and HPE of SLNB for each patient.

Statistical analysis

The data was entered in MS Excel 2010 and statistical analysis was performed with SPSS software version 22. One-sample, Kolmogorov-Smirnov test decided the normality of the data sets. The Parametric tests were used to analyse normally distributed data and Non-parametric tests for non-normally distributed data. The categorical data was analysed with Chi square test. A two by two table (Table 1) of results of FS and HPE (gold standard) was prepared and the following formulae were used for calculation.

Table 1: The two by two table.

| Test		HPE (gold standard test)		Total
		Positive test	Negative test	
FS	Positive test	A	C	A + C
	Negative test	B	D	B + D
Total		A + B	C + D	

- Sensitivity = A/(A + B)
- Specificity = D/(C + D)
- Accuracy = (A + D)/(A + B + C + D)
- Positive predictive value = A/(A + C)
- Negative predictive value = D/(B + D)
- False negative rate = 1-sensitivity
- False positive rate = 1-specificity

Confidence intervals (CI) for sensitivity, specificity and accuracy were calculated with exact Clopper-Pearson CI.

RESULTS

A total of 61 patients with carcinoma breast underwent sentinel lymph node (SLN) identification using 1% methylene blue dye between July 2017 and November 2018 (Figure 1). In one patient SLN was not identified, in further five patients an intra-operative frozen section (FS)

was not performed due to logistic reasons. These patients were excluded from the study.
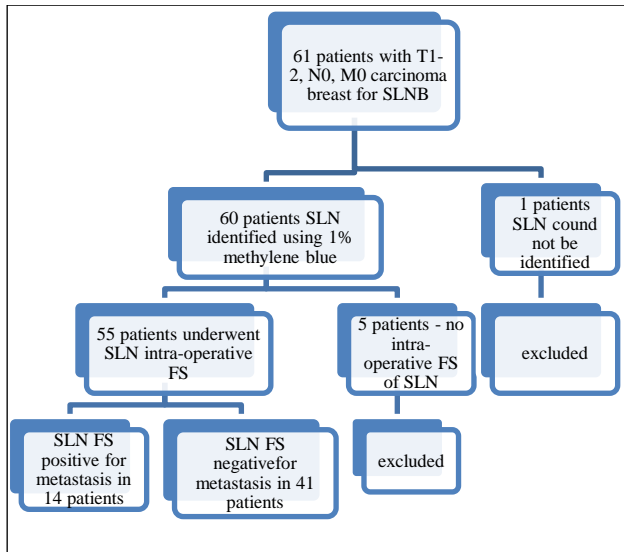
(SLNB-sentinel lymph node biopsy, SLN-sentinel lymph node, FS- frozen section).

**Figure 1: Patients in the study according to CONSORT guidelines.**

In 55 patients an intra-operative FS evaluation of the SLN was performed. All patients were female; the mean age was 53 years (range 30-84±15.09 SD). The age distribution, the side of tumor and quadrant distribution is shown in Table 2. The primary tumor was clinically staged as either T1 (23.6%) or T2 (76.4%); all patients had clinically N0 axilla and no evidence of distant metastasis.

**Table 2: The baseline patient and disease details.**

Base line data of patients (n=55)			
Variables		**Number**	**Percentage**
Age group (years)	30-39	14	25.5
	40-49	10	18.2
	50-59	12	21.8
	60-69	10	18.2
	70-79	5	9.1
	80-89	4	7.3
Side of lesion	left	30	54.5
	right	25	45.5
Quadrant of lesion	central	7	12.7
	inner	10	18.2
	outer	31	56.4
	upper	7	12.7
Clinical Tumor stage	T1	13	23.6
	T2	42	76.4
Clinical Nodal stage	N0	55	100
	N1	0	0
	N2	0	0

All patients underwent surgery for the primary tumor and SLN identification and intra-operative assessment of the
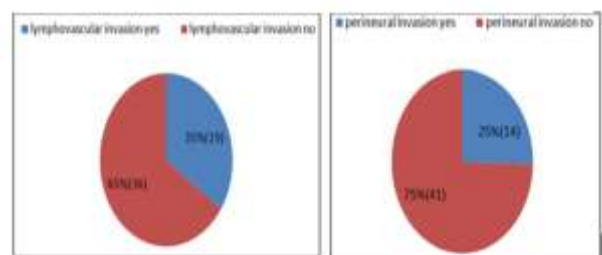
SLN using FS. The patients underwent AD upto the level of highest palpable/enlarged node if - the frozen section was diagnostic of nodal metastasis or if the surgeon found suspicious nodes during the axillary exploration; rest of the patients underwent SLNB only.
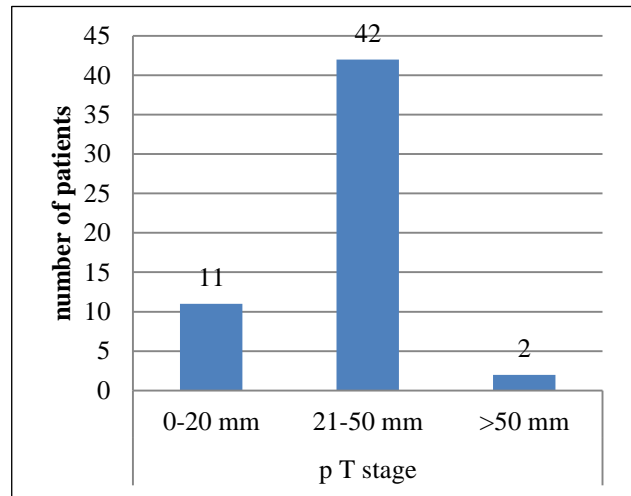
Figure 2: The pathological T stage of the tumor.
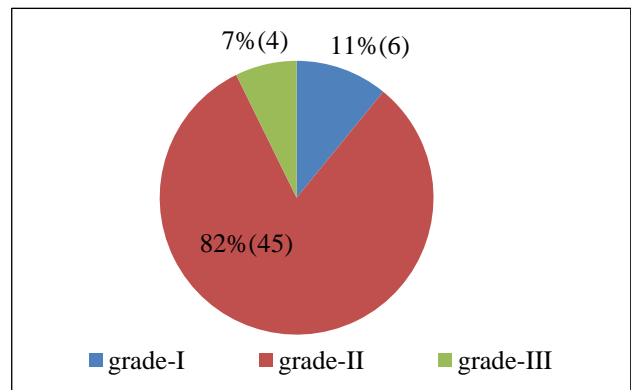
Figure 3: The grade of primary tumor.

Figure 4: Presence of lymphovascular and perineural invasion in the primary tumor.

On histopathology 20%, 76.4% and 3.6% patients had T1, T2 and T3 primary tumors respectively (Figure 2); 10.9%, 81.8% and 7.3% tumors were grade I, II and III respectively (Figure 3); 34.5% tumors demonstrated lymphovascular invasion and 25.5% perineural invasion (Figure 4).

Table 3: The frozen section and histopathology details of sentinel lymph nodes (n=55).

Sentinel lymph node results			
Variables		**Result**	**Range**
Median number of SLN identified		4	1- 14
Mean SLN size (mm)		13.84 (±5.238 SD)	5- 28
		Number of patients	%
SLN size groups (mm)	0-5	3	5.5
	6-10	17	30.9
	11-15	22	40.0
	16-20	8	14.5
	21-25	4	7.3
	26-30	1	1.8
SLN FS positive for metastasis	Yes	14	25.5
	No	41	74.5
SLN HPE positive for metastasis	Yes	16	29.1
	No	39	70.9
Number of SLN positive on FS	1	6	10.9
	2	3	5.4
	3	4	7.3
	9	1	1.8
Number of SLN positive on HPE	1	5	9.1
	2	5	9.1
	3	3	5.4
	4	2	3.6
	9	1	1.8

(SLN- sentinel lymph node, SD- standard deviation, FS- frozen section, HPE- histopathology).

Table-4: The sensitivity and specificity of FS when compared to HPE in SLNB.

SLN FS positive and HPE positive cross tabulation						**Percentage**	**95% CI**
		SLN HPE positive		**Total**	**Sensitivity**	81.25	54.35-95.95
		Yes	**No**		**Specificity**	97.44	86.52-99.94
SLN FS positive	Yes	13	1	14	**PPV**	92.86	64.93-98.92
	No	3	38	41	**NPV**	92.68	82.02-97.24
Total		16	39	55	**accuracy**	92.73	82.41-97.98

(FS- frozen section, HPE- histopathology, SLNB- sentinel lymph node biopsy, SLN- sentinel lymph node, PPV- positive predictive value, NNV- negative predictive value, CI- confidence interval).

Table 5: Cross tabulation of level of axillary dissection with SLN metastatic status.

Axillary dissection level and SLN metastasis cross tabulation							
		SLND FS positive N (%)		Total	**SLND HPE positive N (%)**		Total
		Yes	No		Yes	No	
Axillary Dissection level	SLNB	0	19 (46.34)	19 (34.54)	0	19 (48.72))	19 (34.54)
	II	1 (7.14)	18 (43.90)	19 (34.54)	3 (18.75)	16 (41.02)	19 (34.54)
	III	13 (92.86)	4 (9.76)	17 (30.99)	13 (81.25)	4 (10.25)	17 (30.99)
Total		14	41	55	16	39	55

(SLN- sentinel lymph node, SLNB- sentinel lymph node biopsy, FS- frozen section, HPE- histopathology).

A median of four SLN were identified with a mean size of 13.84 mm (Table 3). On FS out of 55 patients the SLN was positive for metastasis in 14 (25.5%) patients; with 1, 2, 3 and 9 SLN positive in 6, 3, 4 and 1 patients respectively. On HPE out of 55 patients the SLN was positive for metastasis in 16 (29.1%) patients; with 1, 2, 3, 4 and 9 sentinel lymph nodes positive in 5, 5, 3, 2 and 1 patient respectively. The median of 2 SLN were positive for metastasis on FS and HPE in the patients with nodal metastasis.

The Table 4 shows the cross tabulation of the FS and HPE results of SLN identified in the patients. There were 13 true positive and 38 true negative results for FS,

taking HPE as the gold standard test. Three patients had false negative result and one patient had false positive result. The sensitivity, specificity, positive and negative predictive values, false negative and false positive rates were 81.25%, 97.44%, 92.86%, 92.73%, 18.75% and 2.56% respectively in this study. The false negative rate was 18.75% and false positive rate was 2.56%. The overall accuracy of FS of SLN in early carcinoma breast was found to be 92.73%. The 3 patients with negative FS but positive HPE for lymph nodal metastasis in the SLNB were further analyzed and found to have only micrometastasis.

When we looked at the level of AD performed and compared with the SLN metastasis, it was found that in patients with SLN metastasis on FS 92.86% and on HPE 81.25% patients under went level III AD respectively and rest level II AD (Table 5).

DISCUSSION

In patients with early breast cancer SLNB is a precise technique for screening the axillary lymph nodes. Moreover, a negative SLNB for metastasis can prevent the morbidity of a complete ALND.[9-11] Nevertheless, current studies have questioned the need for intra-operative assessment of SLNB, especially in situations when complete ALND may not be done even in the presence of metastasis in SLN. The ACOSOG Z0011 study performed in patients with early breast cancer demonstrated no difference in loco-regional disease recurrence in patients with 1-2 SLN metastasis, who were randomized to either SLNB only or SLNB and ALND; with no inferior survival with the use of SLNB alone compared with ALND in a selective patient population (i.e. patients with clinically negative axilla, tumor size less than 20 mm, and 1 or 2 positive nodes).[12,13]

In our study only 6 (10.9%) patients had 3 or more SLN metastasis on HPE; thus, applying Z0011 criteria 89.1% patients could potentially avoid an ALND if they all had undergone a breast conserving surgery and received post-operative radiotherapy. Overall, in patients with negative SLN on FS (41/55)-46.34% underwent SLNB alone, 9.76% patients underwent a complete ALND and rest a level II ALND (following the study criteria of intra-operative clinically suspicious enlarged nodes); in patients with negative SLN on HPE (39/55)-48.72% underwent SLNB alone, 10.25% patients underwent a complete ALND and rest a level II ALND in our study (Table 4). Some have even further questioned the need of any SLNB evaluation at all, suggesting that the pre-operative axillary ultrasound evaluation of nodes and fine needle aspiration cytology (FNAC) of suspicious nodes could be enough to guide the decision regarding ALND.[14,15]

Other concerns for the doubt regarding need for intra-operative assessment of SLNB are the different methods used (FS vs. touch prep imprint cytology) and the quantity of tissue utilised for intra-operative assessment. A review of literature reveals various techniques for intra-operative assessment of the SLNB.[16] Guidelines published by the College of American Pathologists for the processing of SLNB focus on grossing and processing aspect of the specimen for HPE only, but there is no guideline on preferred technique for intra-operative assessment-either FS or touch prep.[17,18] In a study published in 2012, 126 early breast cancer women with T1 tumors were prospectively enrolled, 221 axillary nodes were assessed intra-operatively with FS and imprint cytology (IC). The sensitivity, specificity and accuracy of FS was found to be 75.7%, 100% and 91.9%; of IC was found to be 70.3%, 98.6% and 89.1%.[19]

Table 6: The comparison of sensitivity of intra-operative frozen section of sentinel lymph nodes on literature review with present study.

Studies	Number of patients	Intraoperative technique	Gold standard used	Sensitivity for SLN metastasis
Turner et al[20]	278	FS	HPE, IHC	98
Weiser et al[21]	890	FS	HPE, IHC	92
Brogi et al[22]	133	FS	HPE, IHC	96
Van de Vrande at al[23]	615	FS	HPE, IHC	71.6
Chao et al[24]	200	FS, IHC	HPE, IHC	68
Grabau et al[25]	108	FS, IHC	HPE, IHC	73
Langer et al[26]	648	FS, IHC	HPE, IHC	98
Celebioglu et al[27]	102	FS, IHC	HPE	73.5
Tille et al[19]	161	FS, IHC	HPE	83.3
Somashekhar et al[28]	164	FS	HPE	92.6
Current study	55	FS	HPE	81.25

(SLN- sentinel lymph node, FS- frozen section, HPE- histopathology, IHC- immunohistochemistry).

Another study from Switzerland assessed 279 axillary nodes from 149 carcinoma breast patients with the intent of assessing the diagnostic accuracy of intra-operative FS evaluation of SLNB for micrometastasis. They found the sensitivity of FS for macrometastasis to be 83.3% and for micrometastasis to be only 40%. The specificity was

100%. On literature search the sensitivity of intra-operative FS of SLNB varied from 68% to 98% for macrometastasis (Table 6), in the current study the sensitivity of 81.25% was well within this range.[19-28]

The large variation in accuracy of intra-operative assessment of SLNB reflects the different techniques being employed for FS analysis. These differences in techniques include-the intervals used for cutting of lymph nodes, number of cut sections analysed and if immunohistochemistry (IHC) was used with HPE as gold standard for comparison, as well as use of perioperative IHC.[27] The NCCN guidelines do not recommend ALND for only micrometastasis or isolated tumor cells on IHC.[3] Thus, we do not see the need for diagnosing these during an intra-operative assessment of SLNB.

With the FS protocol used at our institute of bisecting all sentinel lymph nodes for FS, with 4mm sectioning of bisected nodal tissue for FS and HPE for rest of the bisected nodal tissue, the sensitivity and accuracy in our study was 81.25% and 92.73% respectively. An intra-operative assessment of SLNB can potentially guide the surgical team towards the level of axillary dissection, save the morbidity of complete ALND and a second axillary surgery.

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