



DESCRIPTIVE STUDY OF SENTINEL LYMPH NODES /AXILLARY SAMPLING ON FROZEN SECTION IN CASES OF CARCINOMA OF BREAST

Pathology

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ABSTRACT

Background: The frozen section provides rapid diagnosis to guide intra or perioperative patient management. Intraoperative frozen sections of sentinel lymph nodes can be used to identify metastatic disease, allowing immediate axillary lymph node dissection. **Aims:** To Study Sentinel Lymph node /Axillary Sampling on Frozen Section in Cases of Carcinoma of the Breast and to assess the accuracy of frozen section diagnosis. **Materials and Methods:** - An observational descriptive study was carried out in the department of pathology. A total of 33 patients were evaluated during January 2021 to July 2022. SLNs were examined using standard frozen section procedure and sections were stained with rapid H & E. Patients with positive SLN on FS underwent ALND. The remaining frozen tissue was processed for routine histopathology processing. **Results:** - Out of 33 cases, 9 (27.27%) cases are positive on the frozen section and 24 (72.72 %) cases are negative on the frozen section. However, on the paraffin section, 14 (42.42%) cases are positive and 19 (57.57 %) are negative. There was no false positive case (specificity-100%). 14 cases show tumour deposit in sentinel lymph node. 13 cases had macro-metastatic tumour deposit and 01 case shows micro-metastatic tumour deposit. The diagnostic accuracy is 84.84% and sensitivity 64.28%. **Conclusions:** - Intraoperative frozen section diagnosis of sentinel lymph nodes in cases of carcinoma of breast can identify metastatic disease, allowing immediate axillary dissection, if necessary, thus avoiding the need for reoperation. Arm and shoulder morbidity caused by axillary lymph node dissection (ALND) can be avoided in cases where it was not indicated on frozen section.

KEYWORDS

Frozen Section, Sentinel Lymph Node, SLNB, Breast Carcinoma.

INTRODUCTION

In the 1940s, Gilchrist and Zeidman and Buss demonstrated that metastatic cells spread through regional lymphatics in an orderly and reproducible manner, thus paving way for the evolution of sentinel lymph node biopsy (SLNB). The sentinel lymph node (SLN) is the first node that drains the lymph from a particular organ before draining it into subsequent nodes (non-SLNs). Sentinel lymph node/s existence in the breast was proven by Kett et al. and Krag et al.^(1,2). The frozen section technique was first introduced by the pathologist, William H. Welch, in 1981. Frozen section preparation was made easier in the 1960s using Cryostat, a cabinet with -20° to -30° Celsius cooling and enclosing a microtome blade⁽³⁾. Axillary lymph node status is the most powerful independent predictor of survival and the risk of recurrence in patients with breast cancer. SLNB has proven to be an accurate staging procedure in breast cancer patients and has replaced axillary lymph node dissection (ALND) in the clinically node-negative axilla. Consequently, unnecessary arm morbidity caused by ALND can be avoided. Frozen Section has the advantage of enabling ALND to be performed immediately, thereby avoiding the need for reoperation^(4,5). Sentinel lymph node dissection is a safe technique. It has been proven in multiple studies that SLN dissection lowers the incidence of complications compared to axillary lymph node dissections and better the quality of life than ALND^(6,7,8,9,10). Frozen section analysis had a false-negative rate and the micrometastasis in the SLN was the most important factor associated with it^(11,12,13).

MATERIALS AND METHODS:-

An observational descriptive study was carried out in the department of pathology in collaboration with the department of surgical oncology. A total of 33 patients were evaluated during January 2021 to July 2022, only patients undergoing intraoperative (Frozen Section) procedures for Sentinel Lymph node/ Axillary sampling for carcinoma of the breast.

Inclusion Criteria:

All sentinel lymph nodes/axillary sampling tissue of carcinoma of breast cases sent from operation theatre to the frozen section unit to

Histopathology Laboratory, Pathology Department.

Exclusion Criteria:

1. Inadequate tissue
2. Electricity failure
3. Technical failure
4. Sample received after a delayed period of time
5. Sample received in formalin

Study Procedures:

In all selected patients, peritumoral injection of 1% Methylene blue dye (4ml) at the 3, 6, 9, and 12 o'clock positions were given. The stained nodes were removed initially and sent for frozen section examination.

Table 1. Patient Characteristics

cases	33
Median age (years)	52
Laterality of SLN	
Right	14
Left	19
Tumour size	
T1	2
T2	22
T3	3
Post lumpectomy	6
Nodal status	
N0	10
N1	11
N2	4
N3	2
Post lumpectomy	6
Tumour grade	
II	8
III	19
Post lumpectomy	6

Type of Surgery	
Modified Radical Mastectomy	14
Breast Conservative Surgery	19

In the laboratory, the SLN was initially grossed by the pathologist, each SLN is grossly sectioned parallel to the long axis, one half of sentinel lymph node is used for frozen section and other half for routine paraffin processing. The remaining frozen tissue was processed for making permanent paraffin sections and examined using haematoxylin and eosin staining without additional immunohistochemical staining. Deeper sections were studied on paraffin sections before giving final report in all negative cases. Final analysis was done and the frozen section report was compared with the final histopathology report as to whether the frozen section was concordant/discordant.

The sensitivity, specificity, and positive and negative predictive values of the frozen section were calculated. The accuracy rate of the frozen section was calculated and compared with various studies with the aim of validating frozen section evaluation at our centre. Excised lymph nodes are also submitted for intraoperative evaluation using imprint cytology. The results of present study were based on comparison of a single frozen section and a single permanent section without IHC. We have to check the sentinel lymph nodes by using multi sections and IHC again if resources are available.

RESULTS:

The patient characteristics are mentioned in table 1. The age of the patients ranged between 30 and 85 years. The median age is 52 yrs. A total of 63 SLNs were removed from 33 patients with an average of 1.9 lymph nodes per patient. out of 33 cases, 9 (27.27%) cases are positive (showing tumour deposit) on the frozen section and 24 (72.72 %) cases are negative (without tumour deposit) on the frozen section. Imprint cytology shows the same results as the frozen section. However, on the paraffin section, 14 (42.42%) cases are positive, 13 cases show macro-metastasis and one case shows micro-metastasis (post NACT patient) and 19 (57.57 %) are negative. 5 cases show discordance. In present study, there was one case which was reported atypical or suspicious for malignancy on frozen section, shows predominantly lymphoid cells admixed with histiocytes and few dissociated epithelial appearing cells with nucleomegaly. Drying and ice crystal artefact was noted.

ANALYSIS OF SENSITIVITY, SPECIFICITY RATES AND ACCURACY OF FROZEN SECTION (n=33)

Table 2: Comparison Of Frozen Section Versus Paraffin Section

Frozen section	paraffin section		
	Positive	Negative	Total
Positive	9	0	9
Negative	5	19	24
Total	14	19	33
Sensitivity %	64.28%		
Specificity%	100%		
False-negative rate %	35.17%		
Positive prediction rate %	100%		
Negative prediction rate %	79.16%		
Diagnostic accuracy %	84.84%		

Table 2 shows 9 true positive cases (positive on both FS and paraffin section), 19 true negative cases (negative on both FS and paraffin section) and 5 false negative cases (negative on FS and positive on paraffin section). There was no false positive case (positive on FS and negative on paraffin section).

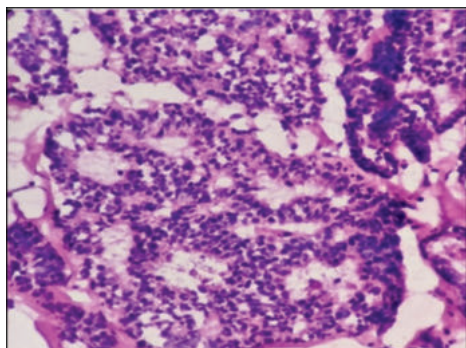


Figure -1 FS showing tumour deposit in sentinel lymph node (H&E, 400x)

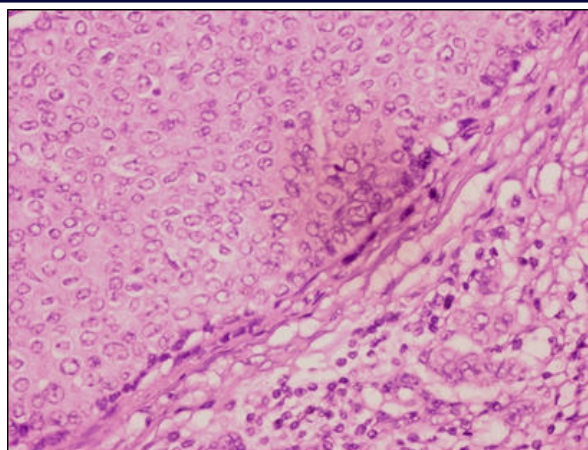


Figure -2 Final paraffin section showing tumour deposit in sentinel lymph node (H&E, 400x)

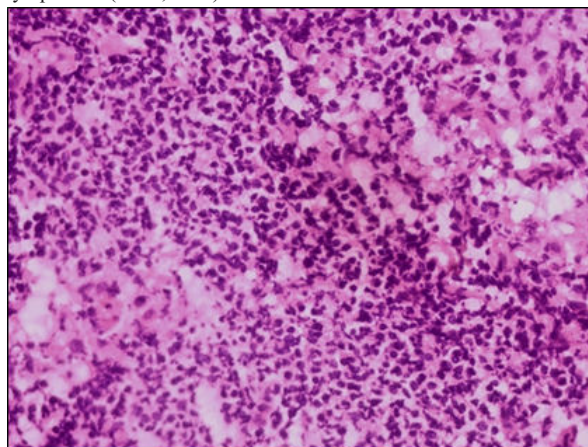


Figure -3 False negative, frozen section showing lymphoid cells. No tumour deposit was seen in the sentinel lymph node (H&E, 400x)

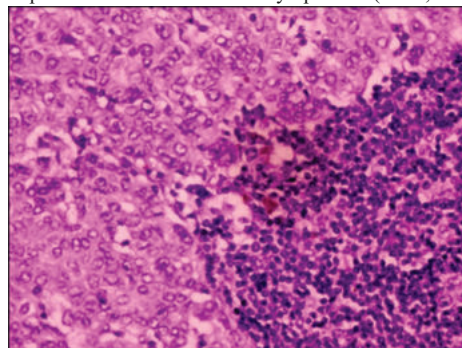


Figure -4 False negative, Final paraffin section showing tumour deposit in the sentinel lymph node (H&E, 400x)

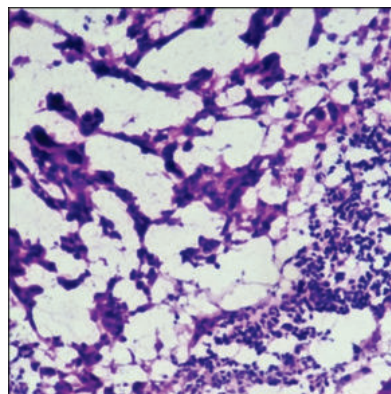


Figure -5 Frozen section showing ice crystal artefact in the sentinel lymph node (H&E, 400x)

DISCUSSION:

Breast cancer is a major health problem and the most common cancer in women. As a staging and therapeutic tool, axillary surgery is traditionally an integral part of locoregional therapy of breast cancer, however, recent trends shifted from more radical axillary lymph node dissection to a less morbid sentinel lymph node biopsy.⁽¹⁾ Intraoperative lymphatic mapping identifies the sentinel lymph nodes which can be submitted as frozen sections for assessment of metastasis⁽⁶⁾. Routine intraoperative FS diagnosis of SLNs can identify metastatic disease, allowing immediate axillary dissection, if necessary, and avoiding the need for reoperation and effectively reduces the number of patients going for second operation. The disadvantages of this procedure are its cost and additional surgical time required for the diagnosis, so the intraoperative diagnosis of the harvested nodes must be performed quickly with highest degree of accuracy possible⁽⁴⁾. SLNB useful in patients undergoing a mastectomy with immediate reconstruction (i.e., deep flap reconstruction)⁽⁹⁾.

This study reveals the usefulness and limitations of the frozen section diagnosis of sentinel lymph nodes in patients with breast cancer. This study evaluated the accuracy of intraoperative FS diagnosis with the widely used conventional paraffin processing. However, the sample size of specimens is limited for FS and there are technical difficulties in getting good quality sections, and staining of tissues which influence the interpretation of the section by reporting pathologists. The importance of identification of micrometastasis is not clear in terms of predicting further nodal involvement, prognosis and indications for adjuvant systemic treatment. It remains unclear that isolated tumour cells reflect metastases capable of further growth and dissemination. Isolated tumour cells and micrometastasis have less prognostic significance than macrometastases⁽³⁾. ACOSOG Z0010 trial showed presence of micrometastases does not change the overall survival in breast cancer^(14,15). Frozen section may fail to detect micro-metastasis in lymph nodes. For micro-metastasis extensive sampling of sentinel lymph node is suggested still confirmation of metastasis is deferred till paraffin sections. Our study suggests that frozen section is more accurate in detection of macro-metastasis.

Methods used were heterogeneous and the interpretation of results consist of any type of nodal involvement as metastasis, so these results are variable. In our institution, all the sentinel lymph nodes received were submitted for evaluation. They were subjected to multilevel sectioning on the initial frozen sections as well as subsequent paraffin tissue blocks until the tissues were exhaustive.

Table 3 - Comparison Of Diagnostic Accuracy, False Negative Rate (FNR) And Sensitivity With Different Studies.

STUDY	ACCURACY (%)	FNR (%)	SENSITIVITY (%)
Noriaki wada et al 2004 (4)	95	16	84
S. P. Somashekhar et al 2013 (5)	98.1	7.3	92.6
Shau-Kong LAI et al 2018 (6)	97.5	13.3	86.7
Yolanda H.Y. Chan et al 2010 (7)	86.4	38.4	61.6
Leonardo Russo et al 2017 (11)	92.88	23.70	76.30
Ambreen Moatasim et al 2012 (16)	96	11	90
Present study 2022	84.84	35.17	64.28

Our intraoperative analysis results are similar to most published data. This study showed the test sensitivity was 64.28 %. A review of the literature showed that the sensitivity of the intraoperative frozen section analysis technique varies between 52% to 89%⁽⁶⁾. Test accuracy in our study was 84.84% and the test was highly specific at 100%. In the present study, there was no false positive case. True positive cases comprised of macro-metastasis. In present study there were 5 false negative cases. False negative cases were reviewed to see any interpretative error and there was no change in the interpretation. In false negative cases, sampling error was noted in which malignant cells were only present in the deeper final paraffin sections and were not seen in initial FS. This is an inherent technical limitation of intraoperative FS. All cases showed metastatic foci within lymph node parenchyma without subcapsular space involvement. Extensive ice crystal and sectioning artefacts are also seen. Knowledge on limitations of FS analysis with careful evaluation of FS specimen will be helpful in reducing interpretation error. To minimize the false positive results, intraoperatively atypical or suspicious cases are considered negative at the time of surgery unless confirmed as positive in permanent section diagnosis. In our study, there was one such case. Atypical or suspicious cases should be confirmed on a permanent

section to avoid unnecessary ALND.

The false negative rate of the present study was 35.17 %. A false negative rate of the frozen section for axillary sentinel lymph nodes ranges from 7 to 56 %^(17,18). A false negative rate is a rate at which metastases of SLNs recognized in the permanent section diagnosis is not recognized in the frozen section diagnosis⁽⁴⁾. Multiple permanent sections examination would probably lead to a poorer FNR. The best FNR should be reported when the final histological evaluation considers only the same surface as that used in the intraoperative assessment. In the present study, there are 5 false negative cases due to sampling error. Chopping and entire submission of sentinel lymph node could minimize this error. Variability in the sensitivity and false negativity are partly due to different practices and protocols in different institution.

The sensitivity of intraoperative evaluation varies between 44 % to 94 %⁽⁵⁾, this variation in sensitivity is due to different histopathological techniques used for frozen section analysis, number of serial sections and proportion of SLN macro-metastases in that group of patients.

In present study, we had also done imprint cytology for all the cases and results were similar to intraoperative frozen section diagnosis. Compared with frozen section imprint cytology was easy to perform with no loss of nodal tissue, available for paraffin section analysis, lower cost and shorter time requirement. However, cytopathological expertise are required. Loss of tissue during Frozen section analysis might impair the probability to detect lymph node metastases. Relationship exists between micro-metastasis and false negativity⁽⁴⁾. Frozen section and touch imprint cytology and for the intraoperative evaluation of sentinel lymph nodes were same; however lower sensitivity of touch imprint in detecting metastasis with higher false-negative rate have been found in various studies done earlier.

CONCLUSION

Intraoperative frozen section diagnosis of sentinel lymph nodes in cases of carcinoma of breast can identify metastatic disease, allowing immediate axillary dissection, if necessary, thus avoiding the need for reoperation. Arm and shoulder morbidity caused by axillary lymph node dissection (ALND) can be avoided in cases where it was not indicated on frozen section.

Intraoperative frozen section analysis for axillary sentinel lymph node is accurate, specific, reliable and safe procedure. False negative intraoperative findings were mainly due to sampling error, ice crystal artefact and drying artefact. Chopping and entire submission of sentinel lymph node could minimize sampling error.

An understanding of limitation of frozen section analysis with careful evaluation of frozen section specimen will be helpful in improving its interpretation. A recommendation to serial sectioning of sentinel lymph nodes that are >5 mm at 2 mm interval and lymph nodes that are <5 mm in size bisected to decrease the error.

In frozen section additional surgical time is required for the diagnosis, so the intraoperative diagnosis must be performed and reported quickly with highest degree of accuracy.

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