

“Intraoperative Touch Imprint Cytology of Sentinel Nodes”, Can it Serve as an Alternative to Frozen Section? – A Prospective Comparative Study of Touch Imprint Cytology and Frozen Section in the Intra Operative Assessment of Nodal Metastasis in Carcinoma Breast.

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Abstract: Sentinel lymph node biopsy (SLNB) is now known to offer an excellent alternative method to routine axillary lymph node dissection in staging of breast cancer. However, this minimally invasive technique requires facilities for frozen section (FS) and intraoperative analysis, which are sadly not available at most centers in India. At the same time Touch imprint cytology (TIC), is relatively less cumbersome technique for intraoperative assessment which may provide a fast and accurate method for intraoperative assessment of sentinel lymph nodes.

Aim: This study was done to assess the accuracy and feasibility of using the TIC as an alternative to FS to assess metastasis in the sentinel node.

Methods: Touch imprint cytological analysis of sentinel lymph nodes was compared prospectively with frozen section and the findings obtained on routine paraffin sections. Touch imprint slides from 32 consecutive women undergoing mastectomies (from Jan 2014 to May 2016) for invasive breast cancer (all 32 were T1-T3 and with clinically N0 nodal status) were prepared during operation from multiple sections of sentinel lymph nodes, stained with haematoxylin and eosin.

Results: The sensitivity of TIC was 89.47% (95% CI, 68.49-98.81%), when compared with the final histopathological result as a gold standard method. The sensitivity of the FS was 94.74% (95% CI 73.97% to 99.87%), when compared to the HPE results. The specificity of the TIC and FS when compared to HPE is 100%.

Conclusion: This study has lent credibility to the possible use of TIC as an assessment tool of the sentinel node in centers where facilities for frozen section are not available and hence expanding the population of potential beneficiaries who can be spared of morbidity associated with axillary dissection

Keywords: Touch Imprint Cytology, Frozen Section, Sentinel Lymph Node biopsy

I. Introduction

In patients with breast cancer, the presence of lymph node metastases is the vital prognostic factor and indicates the need for adjuvant therapy for loco regional disease control. Sentinel lymph node biopsy (SLNB) is a widely used technique for the intraoperative evaluation of axillary lymph nodes when these appear normal on clinical examination. The finding of lymph node metastases can be followed by axillary lymph node dissection (ALND) for local control as well as definitive breast surgery. Conversely, when the sentinel lymph nodes (SLNs) are free of cancer, ALND is not indicated and the risk of complications such as seroma, infection, pain and lymphoedema is significantly reduced [1].

Intra-operative diagnostic modalities are important in making immediate decisions for the optimal extent to surgery. To minimize the extensive axillary dissection, sentinel lymph node metastases is judged by FS and TIC. Although it is established that frozen section is the most reliable intra-operative diagnostic method, but the technique is expensive, requires technical expertise, expensive equipment and is not available in many hospitals in India.

Touch imprint cytology is considered as a good alternative method and it is useful especially in hospitals with limited technical and financial resources [2]. Thus, it is a reliable, rapid, relatively safe and cost-effective technique which requires less financial, human and technical resources. The current study was planned to evaluate the diagnostic accuracy of touch imprint cytology and frozen-section technique which are compared with the gold standard Histopathological examination (HPE).

II. Material and Methods

32 consecutive patients who had a diagnosis of T₁- T₃ invasive breast cancer with clinically negative axillary lymph nodes (Preoperative evaluation of axillary lymph node status was obtained by clinical examination) were considered for this study and Informed consent was obtained from all of them. Patients with stage IV disease and clinically node positive disease were not included in this study. All the enrolled individuals were subjected for lymphatic mapping and sentinel node biopsy (SLNB) was taken.

According to this study design the SLN were identified by using subareolar injection of 10ml of Methylene blue. All identified SLNs were sent to pathology for a detailed gross and microscopic evaluation. SLNs received fresh in the Pathology department Frozen section (FS) suite were bisected to give larger surface area for TIC and microscopic examination. The touch imprints were made of both surfaces of the lymph node sections, fixed in 95% alcohol, and stained by the Hematoxylin and Eosin (H&E) method and sent for examination. The rest were sectioned at 2-mm intervals along the long axis to give larger surface area for microscopic examination and subjected for Frozen Section (FS). The results of FS alone were used to decide whether to complete the axillary lymph node dissection at the time of primary breast surgery or not. The results of TIC were not used in the intraoperative decision-making process. The results of TIC & FS were compared with regular gold standard paraffin section HPE for obtaining statistically significant data.

2.1. Statistical analysis:

The sensitivities, specificities, positive and negative predictive values of TIC and FS for the detection of metastatic tumor in the SLNs were determined with the corresponding 95% confidence intervals (CIs). In the calculations, the data obtained from paraffin section (evaluation by H and E) was considered as the gold standard.

2.2. Results:

In this study, 32 patients with invasive breast cancer were included for study. SLN were identified in 32 patients ranges from 1 through 3 nodes. (Average node yield, 1.59 nodes per patient). Mean age of the patients was 50.25 ± 8.904 years (Range between 30 – 70 years). The quality of the FS and TIC was satisfactory in all patients for histological and cytological interpretation. No male patients included in our study.

TABLE 1: Summary of the comparison between the TIC and the standard HPE

TIC	HPE		
	PARAMETERS	Positive	Negative
	Positive	17	0
Negative	2	13	

TABLE 2: Statistical outcome of TIC Vs HPE

Sl No	Parameters	Formula	Values	95%confidence interval
1.	Sensitivity	TP/TP+FN	17/19 = 89.47%	66.87% to 98.70%
2.	Specificity	TN/TN+FP	13/13 = 100%	75.29% to 100%
3.	Prevalence	TP+FN/Total No of cases	19/32 = 59.38%	40.64% to 76.30%
4.	Positive predictive value	TP/TP+FP	17/17 = 100%	80.49% to 100%
5.	Negative predictive value	TN/TN+FN	13/15 = 86.67%	59.54% to 98.34%
6.	Negative likelihood ratio	100 – sensitivity/ specificity	(100 – 89.4)/100 = 0.11	0.03 to 0.39
7.	Accuracy	TP+TN/Total No of cases	30/32 = 93.75%	----

TABLE 3: Summary of the comparison between the FS and the standard HPE

FS	HPE		
	PARAMETERS	Positive	Negative
	Positive	18	0
Negative	1	13	

TABLE 4: Statistical outcomes of FS Vs HPE

	Parameters	Formula	Values	95% confidence interval
1.	Sensitivity	TP/TP+FN	18/19 = 94.74%	73.97% to 99.87%
2.	Specificity	TN/TN+FP	13/13 = 100%	75.29% to 100%
3.	Prevalence	TP+FN/Total No of cases	19/32 = 59.38%	40.64% to 76.30%

4.	Positive predictive value	TP/TP+FP	18/18 = 100%	81.47% to 100%
5.	Negative predictive value	TN/TN+FN	13/14 = 92.86%	66.13% to 99.82%
6.	Negative likelihood ratio	100 – sensitivity/ specificity	(100 – 94.74)/100 = 0.05	0.01 to 0.35
7.	Accuracy	TP+TN/Total No of cases	31/32 = 96.88%	----

In our study, Sensitivity, Specificity, Positive predictive value (PPV), and Negative predictive value (NPV) have been calculated separately based on the number of the sentinel lymph nodes involved. The sensitivity of TIC was 89.47% (95% CI, 68.49-98.81%), when compared with the gold standard histopathological examination. The sensitivity of the FS was 94.74% (95% CI 73.97% to 99.87%), when compared to the HPE results. The specificity of the TIC and FS when compared to HPE is 100%. The accuracy parameters of TIC Vs FS were 93.75% Vs 96.88%. Frozen section is superior compared to the Touch imprint cytology; however touch imprint cytology may be used as an alternative to frozen section in the places where FS is not available.

III. Discussion

Accurate evaluation of the sentinel lymph nodes intra operatively permits the management of the axillary nodes appropriately. ALND to be performed during the initial operation if the node is positive, saving the patient both the time, cost. It also helps in the assessment of prognosis and loco regional control. The well accepted and currently followed frozen section technique is not readily available everywhere. In India many centers without the FS facilities will routinely do MRM even for early breast carcinoma. This leads to unnecessary lifelong morbidity and mortality associated with axillary dissection. Our study aims at establishing the accuracy and the feasibility of TIC (Cheaper and technically simpler method) compared to FS, and whether it can serve as an alternate tool in the intraoperative assessment of the nodal involvement in carcinoma breast.

Franco lumachi *et al* observed that Imprint cytology alone accurately predicts final LN status in up to 85% of patients, according to tumor type and stage [3]. When compared with permanent histology, the specificity of FS is close to 100%, but the technique remains expensive, labor-intensive and operator-dependent. Furthermore, the process of preparing FS leads to tissue loss, which could result in an under staging of the disease. Using cytological techniques, such as TIC and scrape cytology avoids this. The cut surface of the LN is pressed or scrape on to a glass slide, stained and examined. However, the difference in total charges between the FN and TP groups outweighs the cost of IC, potentially reducing hospital stay and sparing the patient a second operation, and thus IC represents a cost-effective evaluation of patients with Breast cancer.

Andrew *et al* reported that the sensitivity of imprint cytology was 53%, specificity was 98%, positive predictive value was 94%, negative predictive value was 82% and accuracy was 84% [4]. The sensitivity for detecting macrometastases (more than 2mm) was significantly better than for detecting micrometastases (<2 mm), 81 *versus* 21%, respectively ($P < 00001$). The sensitivity and specificity of imprint cytology are similar to that of intraoperative frozen section evaluation. Imprint cytology is therefore a viable alternative to frozen sectioning when intraoperative evaluation is required.

Akbar *et al* reported that the sensitivity of TIC compared with the final histopathological result, considered the gold standard, was 90% (CI, 68.49–98.81%). Similarly, the sensitivities of frozen sections and permanent were the same respectively [5]. The specificities of TIC, FS, and permanent method were 100% (CI, 94.95–100.00). Variations in patient selection criteria, experience of the pathologist, skill of the technician submitting specimen for intraoperative evaluation, and tumor size are important variables that influence the results.

Tew *et al* calculated the sensitivity of four different studies that compared TIC with FS. They found sensitivity of 62% and 76% for TIC and FS respectively [5]. They reported a sensitivity range of 44--100% and a specificity of 100%, including all analytical techniques.

The lowered sensitivity of TIC usually is caused by sampling, in that the metastasis is uncovered after serial cutting of the tissue block. The majority of the previous studies that directly compared TIC with FS analysis in SLNs arrived at similar conclusions. To our knowledge, to date, only Motomura *et al.* have reported that TI cytology was better than FS. The experience of the pathologist, skill of the technician submitting specimen for intraoperative evaluation, and tumor size are important variables that influence the results [5]. Sensitivity for SLN biopsy increased as the tumor size increased and decreased as the proportion of micro metastasis increased

Safina *et al* reported that the sensitivity, specificity, positive predictive value, negative predictive value and true positives of TIC were 96.72%, 100%, 100%, 88.24% and 96.72% respectively [2]. The corresponding values of the FS were 100% each. The diagnostic accuracy calculated for touch imprints and frozen section was 97.37% and 100% respectively. The diagnostic accuracy of frozen section was better than touch imprint cytology in rapid intraoperative diagnosis. However, touch imprints can safely be used as an alternative intra-operative procedure to ensure satisfactory results.

IV. Conclusion

Touch imprint cytology has the added advantage of simplicity, rapidity and not sacrificing tissues. So it is possible to use TIC as a useful screening test in determining lymph node metastasis. If TIC is positive, considering the high specificity of this technique, the surgeon can perform LN dissection simultaneously without any need for FS. If the TIC is negative, FS can be performed as well. With this protocol, unnecessary FS will not be performed. However, in usage of TIC as a screening method, the most important factor is ability of the pathology lab to provide TIC and – if necessary – FS after that. This needs a significant skill of the technicians for preparing samples and also experience of the pathologist and coordination between pathology and surgery department. Thus TIC is used as a alternative to FS in remote centers where facilities for FS not available.

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