



Intraoperative Evaluation of Sentinel Lymph Nodes by Touch Imprint Cytology Technique in Breast Cancer Patients

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Authors' contributions

This work was carried out in collaboration between all authors. Author RO designed the study and performed the operations. Author AJ wrote the manuscript and managed the analyses of the study. Authors AM and PA reviewed the specimens and reported the pathology result. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Axillary lymph nodes evaluation plays an important role in breast cancer surgery. Today and in the sentinel lymph node biopsy era, lymph node metastasis is evaluated by various methods such as Frozen section and Touch imprint cytology each having its pros and cons. Selecting proper method depends on many factors, especially instrument availability. This study was designed to evaluate Touch imprint cytology and Frozen section sensitivity and specificity in detecting sentinel lymph nodes metastasis in breast cancer patients.

Study Design: In a prospective study in 198 early stage (stages 0, 1, 2) breast cancer patients, sentinel lymph nodes (SLNs) were evaluated by Frozen section (FS) and Touch imprint cytology (TIC).

Place and Duration of Study: Department of Surgery and Pathology, Parsian Hospital Tehran-Iran, between 2009 and 2012.

Methodology: In 198 early stage (stage 0,1, 2) breast cancer patients, sentinel lymph nodes (SLNs) were evaluated by Frozen section (FS) and Touch imprint cytology (TIC).
Results: In 126 metastatic lymph nodes of 69 patients, sensitivity and specificity of FS was 89.5% and 99% respectively while 78% and 99.5% in TIC method.
Conclusion: TIC has acceptable accuracy in detecting SLN metastases in breast cancer patients.

Keywords: Sentinel lymph node biopsy; touch imprint cytology; breast cancer; intraoperative pathologic evaluation; frozen section.

1. INTRODUCTION

Evaluating axillary lymph nodes is one of the important parts of breast cancer surgery in order to predict the prognosis, to improve local control and to determine the candidates for adjuvant treatments [1].

In the past two decades, routine axillary dissection is replaced by sentinel node biopsy (SLNB) mainly because of considerable morbidities and complications associated with complete axillary dissection [2-4].

Also, intraoperative determination of axillary lymph nodes can preclude the patient from a second operation [5]. There are various methods of evaluating SLN such as frozen section (FS), touch imprint cytology (TIC) and immediate Immunohistochemical (IHC) staining, which all are different in speed, reliability and cost, but up to now, there is no consensus about the best among them to choose [5-7].

FS is expensive, time consuming and requiring specific instruments. Also, FS process causes some tissue loss that can interfere with final pathology result [3]. In contrast, TIC is a fast, inexpensive, easy and widely available method.

The results of studies comparing FS and TIC vary widely, showing sensitivities of 44-100% and 34-95% for FS and (TIC), respectively [2,8-10].

Due to wide variation in TIC results and limited facilities for investigation in Iran, this study is conducted to determine the sensitivity and specificity of TIC in comparison with routine FS analysis in sentinel lymph node (SLN) biopsy in breast cancer patients.

2. MATERIALS AND METHODS

In a prospective study between 2009 and 2012, SLN biopsy was performed in 198 early stage (stage 1,2) breast cancer patients using blue dye (B) or combination of Technetium-blue dye (TC-B) injection. All patients received two periareolar injection of 10-20 MBq 99m Tc-sulfur colloid and one periareolar intradermal injection of 0.5cc isosulfane blue, except patients with upper outer quadrant tumors in whom only blue dye was used for SLN identifications.

SLNs were successfully identified in all patients and were sent for pathologic examination, immediately. Each node was bisected along the long axis, while the cut surface was scrubbed on a glass slide. These slides were then room-dried, stained with toluidine blue,

and analyzed under a microscope. Then, frozen section examination of the nodes was performed by the first pathologist and results were reported to the surgeon.

The results of FS were used as a reference for performing complete axillary dissection. The specimen was then fixed in 10% formalin for routine processing and final pathology review.

TIC slides were reviewed in another section by the second pathologist, while the reviewer was not aware of the FS results.

The sensitivity and specificity of FS and TIC were determined using the permanent pathology result as the gold standard.

The results were analyzed using Statistical Package for the Social Sciences (SPSS), version 16 (SPSS Inc., Chicago, USA).

3. RESULTS AND DISCUSSION

In a three-year period (2009–2012), SLN biopsy was performed for 198 female with breast cancer, and subsequently, 345 SLNs nodes were analyzed. Patients and tumor characteristics are summarized in Table 1.

Table 1. Patients and tumor characteristics

Mean age (years)	49 (24-77)
Histologic type	
Invasive ductal carcinoma	164 (83%)
Invasive lobular carcinoma	18 (9%)
High grade DCIS	11 (5.5%)
Tubular carcinoma	2 (1%)
Medullary carcinoma	1 (0.5%)
Mixed tubular-lobular carcinoma	2 (1%)
Tumor size	
Tis	11 (5.5%)
T1	101 (51%)
T2	81 (41%)
T3	5 (2.5%)
Final pathologic stage	
0	11 (5.5%)
1	80 (40%)
2	109 (54.5%)
SLN detection technique	
Blue dye	70 (35.5%)
Blue dye-Technetium	128 (64.5%)
Mean SLN numbers per patients	1.74 (1-6)

Our results revealed that 126 metastatic nodes were found in 69 patients. Sensitivity and specificity of FS and TIC methods were determined with the final permanent pathology result as the gold standard. These results are shown in Table 2. From 126 positive nodes detected to harbor metastases, 113 were found by FS with the sensitivity of 89.5%, and 99 nodes were found by TIC with the sensitivity of 78%.

Five SLNs had micro-metastases as follows: One was detected only by FS, one was detected only by TIC, two were detected by both methods, and the last one was detected by neither.

Table 2. Comparison between frozen section (FS) and touch imprint cytology (TIC) methods (Total SLNs number = 345)

	FS	TIC
Sensitivity	89/5%	78.%
Specificity	99%	99.5%
Positive predictive value	98%	99%
Negative predictive value	94.5%	89%

Of the 27 false negative results for TIC, 23 nodes were in patients with invasive ductal carcinoma, two were in patients with invasive lobular and two were in patients with a mixed lobular – tubular carcinoma. From 13 false negative results in FS method, one belonged to a patient with invasive lobular carcinoma (ILC) and twelve belonged to patients with an invasive ductal pathology report.

There were 3 false positive in FS and 1 in TIC results in tow patients SLN evaluation.

Today, SLN biopsy has become the standard of care in the evaluation of axillary lymph node status in breast cancer. Although ACOSOG Z11 and other recent studies have showed that SLN biopsy procedure without axillary lymph node dissection (ALND) results in low locoregional recurrence, ALND continues to be strongly recommended in the management of the SLN-positive axilla. Intraoperative information regarding nodal status has important implication for patients who need re-hospitalization for ALND, and also for patients who desire immediate breast reconstruction. Due to the limitations of FS for examination of SLNs such as high costs, time consumption and tissue loss, TIC was introduced as an alternative method [9,11].

In this study, we performed SLN biopsy in 198 patients and 345 nodes have been subsequently examined by FS and TIC methods. The sensitivity of TIC in our study was 78% per node analysis. Previous studies conducted in the 90's to determine sensitivity of TIC had different sensitivity and specificity between 55-62% and 95-97%. It is possible that different types of staining methods show the different results for sensitivity analysis among these studies [12,13].

In one study, Shiver et al. [1] reviewed 132 breast cancer patients and a total of 277 lymph nodes. They reported a sensitivity of 61% and specificity and positive predictive value of 100%. Negative predictive value was 94%. They also said that intraoperative TIC preserved all lymphatic tissue for subsequent analysis, followed by a decrease of 14.7% in need for reoperation in patients and a decrease of 7.2% in operating time [1].

Similar to our obtained result, the time of intraoperative evaluation by TIC was one fifth of time of frozen analysis.

Tew et al. [9] reviewed 31 studies with qualified methodology in a meta-analysis. A pooled sensitivity of TIC was 63% (varied from 34 to 95%) and specificity was 99%. They also reported a sensitivity of 44% to 100% for FS. The specificity for both methods was between 94%-100%. The sensitivity was 22% and 81% for micro-metastases and macro-metastases,

respectively. Finally, they concluded that TIC is a rapid and an inexpensive method for intra-operative SLN evaluation, especially for macro-metastases [9]. The heterogeneity of these studies is explained by a different inclusion criteria and a different cytological technique. In two previous studies, from Mashhad and Shiraz, Iran, the sensitivity of TIC was 71.4% and 90%, respectively [14]. In second study including 40 breast cancer patients and 100 SLNs, intra-operative TIC, FS and cytokeratin immunostaining were compared. They reported a sensitivity of 90% for TIC and specificity of 100% for TIC and FS. They had no false positive results for TIC [14,15]. Although in some previous studies, it has been argued that TIC is less accurate in diagnosis of SLN metastases in ILC, recent investigations have shown that there is no difference between invasive ductal carcinoma (IDC) and ILC in TIC accuracy [7,16].

Nodal metastasis of lobular carcinoma appears as small, regular, and round cells, making them difficult to distinguish from normal nodal cells. In our study, the numbers of ILC specimens were not enough in order to have safe conclusion, but there was no difference in sensitivity and specificity of TIC between invasive ductal and lobular carcinoma.

Many studies have shown that TIC accuracy is low for detection of micro-metastases in SLNs [1]. The average sensitivity of micrometastasis detection using the cytological method was 22% in Tew's meta-analysis. The analysis and sampling technique are the principal factors of variation in the nodal micrometastasis detection rate. In the present study, there were five patients with micro-metastases detected in permanent pathology exam, which is lower than the average number of micro-metastases detection in the same studies. It could be due to low cut numbers of SLNs as shown in some previous studies [5,16]. A study on 552 breast cancer patient from China compared the sensitivity of TIC and one-step nucleic acid amplification (OSNA). The sensitivity and specificity of OSNA were 87.8% and 89.6% respectively compared with those of TIC, which were 81.3% and 96.9%. More micrometastasis-involved nodes were detected by OSNA in comparison with TIC (52.8% vs 25.0%, $P=0.029$).

There was no false positive result in TIC in this study as it was shown in previous investigations. It means that if TIC was used as a guide, none of the patients would have to undergo unnecessary axillary dissection. For the false negative results, if the SLN had micro-metastases, complete axillary dissection could be omitted in selected cases according to patients and tumor characteristics. It is noted that if the permanent pathology report revealed macro-metastases, the patient should undergo axillary dissection in a second stage surgery.

By conducting similar studies and finding the specificity and sensitivity of TIC in each country and also based on the results of other studies, it is possible to replace FS by TIC in evaluating axillary SLNs. It is a fact that many medical centers in developing countries and also some in developed ones have no access to FS equipment. Thus substituting a simpler and more cost effective method with the same specificity and sensitivity, lets the surgeons not to omit intraoperative SLN evaluation from their practice.

In addition it is not only a faster method than FS, but also leads to less tissue loss in pathology specimen.

The limitation of this study in addition to small sample size is the low percentage of micro-metastasis detection that could be due to a technical problem probably the low cut numbers of SLNs or patients' characteristics.

4. CONCLUSION

Finally, it can be concluded that TIC shows an acceptable accuracy in diagnosis of SLN metastases in breast cancer patients and it can be a proper alternative for FS, because it is faster and causes less tissue loss.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shiver SA, Creager AJ, Geisinger K, Perrier ND, Shen P, Levine EA. Intraoperative analysis of sentinel lymph nodes by imprint cytology for cancer of the breast. *American Journal of Surgery*. 2002;184(5):424.
2. Richards ADM, Lakhani SR, James DT, Ung OA. Intraoperative imprint cytology for breast cancer sentinel nodes: is it worth it? *ANZ Journal of Surgery*; 2012.
3. Clarke D, Leung E, Chachlani N, Rowlands D, Simon J, Hero I, et al. Intraoperative assessment of sentinel node using imprint cytology. *World Journal of Surgery*. 2010;34(1):55-61.
4. Schrenk P, Rieger R, Shamiyeh A, Wayand W. Morbidity following sentinel lymph node biopsy versus axillary lymph node dissection for patients with breast carcinoma. *Cancer*. 2000;88(3):608-14.
5. Khoury T, Tan W, Edge S. Accuracy of Touch Preparation versus Frozen Section for Intraoperative Diagnosis of Sentinel Lymph Node Metastases in Breast Cancer with Emphasis on Micrometastases. *North American Journal of Medicine and Science* Jan. 2012;5(1):13.
6. Khanna R, Bhadani S, Khanna S, Pandey M, Kumar M. Comparison of Immunohistochemistry with Conventional Histopathology for Evaluation of Sentinel Lymph Node in Breast Cancer. *Indian Journal of Surgery*. 2011;73(2):107-10.
7. Creager AJ, Geisinger KR, Perrier ND, Shen P, Shaw JA, Young PR, et al. Intraoperative imprint cytologic evaluation of sentinel lymph nodes for lobular carcinoma of the breast. *Annals of Surgery*. 2004;239(1):61.
8. Cox C, Centeno B, Dickson D, Clark J, Nicosia S, Dupont E, et al. Accuracy of intraoperative imprint cytology for sentinel lymph node evaluation in the treatment of breast carcinoma. *Cancer Cytopathology*. 2005;105(1):13-20.
9. Tew K, Irwig L, Matthews A, Crowe P, Macaskill P. Meta-analysis of sentinel node imprint cytology in breast cancer. *British Journal of Surgery*. 2005;92(9):1068-80.
10. Guidroz JA, Johnson MT, Scott-Conner CEH, De Young BR, Weigel RJ. The use of touch preparation for the evaluation of sentinel lymph nodes in breast cancer. *The American Journal of Surgery*. 2010;199(6):792-6.
11. Rubio IT, Korourian S, Cowan C, Krag DN, RNP MC. Use of touch preps for intraoperative diagnosis of sentinel lymph node metastases in breast cancer. *Annals of Surgical Oncology*. 1998;5(8):689-94.
12. Motomura K, Nagumo S, Komoike Y, Koyama H, Inaji H. Accuracy of imprint cytology for intraoperative diagnosis of sentinel node metastases in breast cancer. *Annals of Surgery*. 2008;247(5):839-42.

13. Bahram Memar, Ramin Sadeghi, Narjes-Khatoun Ayati, Seyed Amir Aledavood, Ali Tghizadeh, Shahram Naseri, et al. The value of touch imprint cytology and frozen section for intra-operative evaluation of axillary sentinel lymph nodes. *Pol J Pathol.* 2010;3:161–165.
14. Akbar Safai, Ali Razeghi, Ahmad Monabati, Negar Azarpira, Abdolrasoul Talei. Comparing touch imprint cytology, frozen section analysis, and cytokeratin immunostaining for intraoperative evaluation of axillary sentinel lymph nodes in breast cancer. *Indian J Pathol Microbil.* 2012;55:183-6.
15. Van Diest P, Torrenge H, Borgstein P, Pijpers R, Bleichrodt R, Rahusen F, et al. Reliability of intraoperative frozen section and imprint cytological investigation of sentinel lymph nodes in breast cancer. *Histopathology.* 1999;35(1):14-8.
16. Howard-McNatt M, Geisinger KR, Stewart JH, Shen P, Levine EA. Is intraoperative imprint cytology evaluation still feasible for the evaluation of sentinel lymph nodes for lobular carcinoma of the breast? *Annals of Surgical Oncology.* 2012;19(3):929-34.

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