Original Research

Accuracy of Touch Preparation versus Frozen Section for Intraoperative Diagnosis of Sentinel Lymph Node Metastases in Breast Cancer with Emphasis on Micrometastases

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The sensitivity of intraoperative diagnosis of sentinel lymph node (SLN) metastases in breast cancer is variably low. The purpose of this study was to review the pros and cons of frozen section (FS) and touch preparation (TP) methods, particularly in micrometastases. Intraoperative TP or FS was performed on the SLN of consecutive breast cancer patients from 2007 to 2009. Sensitivity, specificity, and overall accuracy of detecting positive SLNs were calculated for FS and TP groups. There were 396 patients with SLN biopsy. 124 (31.3 %) patients had at least one positive SLN. A total of 1270 lymph nodes were examined intraoperatively, 133 with FS and 1137 with TP. FS was significantly more sensitive than TP, 82.6% and 49.6%, respectively (p<0.0001). There were a total of 57 SLNs with micrometastases. FS was performed on 10 and TP on 47. The sensitivity of FS was 50% and for TP, 19.3% (p<0.0001). Of the 10 positive SLNs using FS, 3 were negative on permanent sections (PS). We conclude that FS is superior to TP as a method of detecting micrometastases in SLNs. However, a significant subset of patients who had positive SLNs on FS became negative on PS. This raises the possibility that some negative SLNs on FS might have been understaged.
[N A J Med Sci. 2012;5(1):13-19.]

Key Words: *sentinel lymph node, breast cancer, micrometastases, frozen section, touch preparation*

INTRODUCTION

The axillary lymph nodes status is the most important factor in determining the prognosis of patients with invasive breast cancer. [1,2] Sentinel lymph node (SLN) biopsy is the preferred procedure for the evaluation of axillary node status in breast cancer patients. Intraoperative diagnosis of SLN metastases facilitates definitive axillary staging because it allows the surgeon to perform a completion axillary dissection during the same surgery if a SLN is found to be positive for metastatic cells.[3,4] Frozen section (FS) and touch preparation (TP) are commonly used for intraoperative evaluation of the SLN. It has been reported that FS is superior to TP in detecting tumor metastases.[5,6]

Although patients with node-negative breast cancer have an excellent prognosis, up to 25% to 30% of these patients will develop local recurrences or distant metastases within 10 years.[7,8] Studies have suggested that this unfavorable outcome may be due in part to undetected occult metastases in the lymph nodes.[9-14] It has been long recognized that evaluation methods have failed to identify all metastatic foci in lymph nodes. Several methods have been proposed to increase the detection rate of axillary metastases.[15-20] Ideally, to detect all positive SLNs, blocks should be serially sectioned until no tissue is left in the block. However, this approach is physically and financially non-feasible.

Another theoretical possibility of occult tumor occurrence is tissue depletion during FS preparation for small tumor metastases. Therefore, in this study we reviewed all SLNs that had intraoperative diagnoses and correlated the histologic findings and metastases size with the permanent section (PS).

METHODS

Cases selection:

A series of breast carcinoma cases with available SLN biopsy examined intraoperatively and with final diagnosis were collected from the archives of pathology department at Roswell Park Cancer Institute between 2007 and 2009. Clinicopathologic variables were collected including patients' age, tumor size, histologic type, Scarff Bloom Richardson (SBR) grade, multifocality and hormone receptor status.

Received 11/30/2011; Revised 1/5/2012; Accepted 1/5/2012
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Sentinel Lymph Node Biopsy Technique:
The SLN biopsy technique in RPCI has previously been explained. [21] The procedure has been revised over the last 5 years based on published experience with intradermal and subareolar injection. 1 millicurie of Tc-99m-sulfur colloid is injected in the skin at the areolar border and under the areola about 20 minutes prior to the skin incision. The axilla is then examined with a gamma probe (Neoprobe 2000, Neoprobe Corporation, Dublin, OH or Navigator, US Surgical Corporation, Norwalk, CT). This method is supplemented with 3-5 cc of 1% isosulfan blue (Lymphazurin) or methylene blue in cases where the sentinel node(s) is not easily identified transcutaneouly with the gamma probe and the breast is massaged for 5 minutes. Intraoperatively, sentinel nodes are identified by the presence of concentration of the radioactive material or the blue dye and excised for pathological examination. Retrieved sentinel nodes are sent fresh for intraoperative pathological examination.

Frozen section and touch preparation evaluation:
All SLN biopsies were grossly examined and serially sectioned with each section measuring ~2-mm thick. In general, TP is the standard procedure in our institution. However, occasionally, FS was elected because the tumor was grossly visible or due to pathologist preference.

When TP was elected as a method of SLN examination, each level of the serially sectioned SLN was touched by a glass slide to prepare the TP slide. One or two TP slides were stained with standard hematoxylin and eosin (H&E), Wright Giemsa (WG) or both, based on the pathologist's preference.

When FS was elected as a method of SLN examination, tissue was embedded in Optimum Cutting Temperature media and cut in Cryostat (Leica CM1850). First, the tissue was leveled to expose the full section of the tissue. The tissue thickness that was cut to level the tissue section was estimated to be between 0.05-mm to 0.5-mm. Two sections were prepared, about 25-µm to 30-µm apart.

The SLN biopsies were evaluated intraoperatively by 13 pathologists. The final PS slides were evaluated by two breast pathologists. Data was extracted from the pathology reports for both intraoperative and final diagnosis. All positive SLN (TP, FS or both) slides were re-reviewed by one pathologist. Metastases size on both FS and PS were recorded. Metastases were classified according to the AJCC TNM Cancer Staging Manual, 7th Edition.[22] Greater than2.0-mm was used as a cutoff for macrometastases, while less than 0.2-mm was used as a cutoff for isolated tumor cells (ITCs). Micrometastases fell between these two values (0.2-mm to 2.0-mm). If the largest cluster was smaller than 0.2-mm, the number of tumor cells should be >200 to be considered micrometastasis, otherwise it was considered ITC. When there was a difference in metastases size between FS and PS, the larger tumor metastases was considered the final size. When SLN was reported positive on FS and negative on PS, both slides were re-reviewed.

Final pathology examination:
Three levels of formalin fixed paraffin embedded SLN biopsies were prepared for PS examination. They were cut with 150 µm interval. Then, there were stained with H&E. Immunohistochemistry using cytokeratin (AE 1/3) was performed only when there was suspicion of metastases on H&E.

Statistical analysis:
Two analyses were carried out, one was patient based and the other was node based. Descriptive statistics such as frequencies and relative frequencies were computed for categorical variables. Numeric variables were summarized using simple descriptive statistics such as the mean, standard deviation, median, range, etc. Fisher's exact test was used to study the association between categorical variables. Trend test was used for ordinal variables. The Wilcoxon rank sum test was used to compare the groups in regards to numeric variables. A 0.05 nominal significance level was used in all testing. All statistical analyses were done using SAS, version 9.1, statistical software (SAS Institute, Cary, NC).

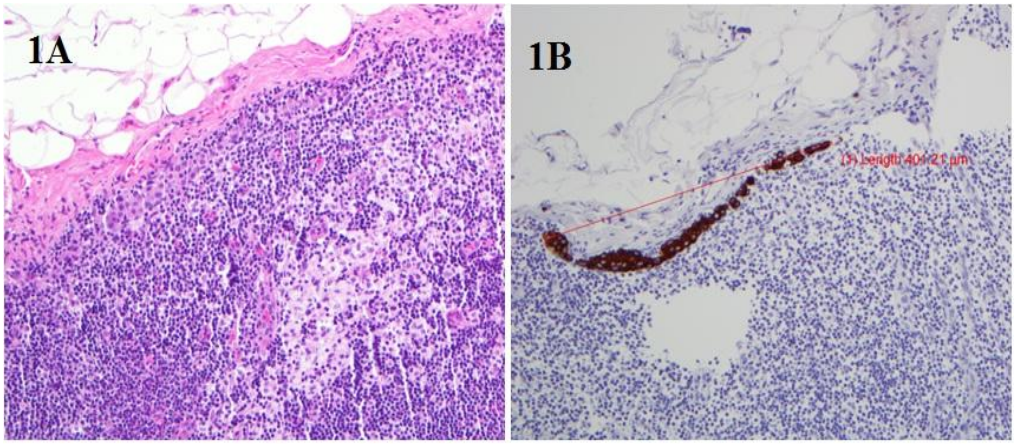

Figure 1. Positive lymph node. **A**, H&E stain showing subcapsular tumor metastases (arrows, 20x). **B**, corresponding cytokeratin (AE 1/3) stain showing tumor that measures about 0.4-mm (micrometastases) (20x).

RESULTS

Clinicopathologic results:

There were 396 patients with SLN biopsy performed. 1270 SLNs were examined intraoperatively by TP or FS. **Table 1** summarizes the clinicopathologic variables of studied patients in correlation with final SLN status. As expected, larger tumor size/stage and tumor multifocality significantly correlated with positive lymph nodes. Cytokeratin AE1/3 confirmed suspicious tumor metastases seen on H&E sections (**Figure 1**).

Results of intraoperative SLN examination, patient based:

There were 396 patients with at least one SLN examined intraoperatively (range of 1 to 12 and median of 4). There were 124 (31.3%) patients with at least one positive SLN. The sensitivity of FS was superior to TP (89.4% and 54.5%, respectively, $p<0.0001$). The difference in overall accuracy between TP and FS was relatively small (93.2% and 89.2%, respectively), due to the high number of true negative cases (**Table 2**).

Results of intraoperative SLN examination, node based:

A total of 1270 SLNs were examined intraoperatively. FS was performed on 133 SLNs and TP on 1137. A total of 171 (13.5%) SLNs were positive. The sensitivity of FS was superior to TP in detecting tumor metastases regardless of metastases size (82.6% and 49.6%, respectively, $p<0.0001$). The difference in overall accuracy between FS and TP was small (92.5% and 95% respectively), due to the relative high number of true negative cases (**Table 3**).

Frozen section versus touch preparation in detecting micrometastases and macrometastases:

There were 9 (5.3%) SLNs with ITC, 48 (28.1%) with micrometastases and114 (66.6%) with macrometastases. After excluding SLN with macrometastases, there were 85 SLN examined by FS (75 negative and10 positive) and 1071 by TP (1024 negative and 47 positive). FS was able to detect 5 of 10 (50%) positive SLNs, while TP was able to detect 11 of 57 (19.3%) ($p<0.0001$) (**Table 4**). The sensitivity for FS vs. TP in detecting macrometastases was not statistically significant [43/48 (89%) vs. 50/66 (75.8%)].

Metastases size correlation between FS and PS:

The tumor size on FS and PS was highly correlated (Spearman correlation coefficient 0.87, $p<0.0001$). However, there were 4 SLNs that were negative on PS but positive either on TP, FS, or both (Table 5). An additional SLN had macrometastases on FS (3.0-mm) but became micrometastases on PS (1.5-mm).

DISCUSSION

This study is retrospective, non-controlled, and had bias in case selection, where FS was performed on cases that were grossly positive. This would falsely increase the number of true positive SLNs by FS compared to TP. Therefore, the comparison between TP and FS for all cases was biased and was not reliable. However, for micrometastases, the tumor metastases were grossly not visible and performing FS was totally random (pathologist preference). Therefore, we think that the comparison is valid with no bias.

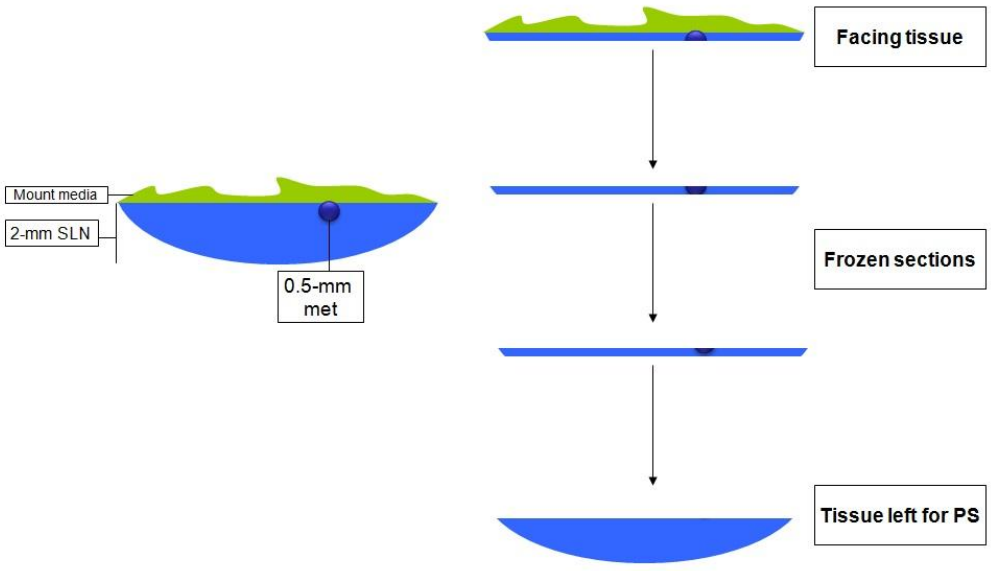

Figure 2. possible scenario explaining the "occult tumor metastases" phenomenon due to FS and PS preparation.

Table1. Clinicopathologic variables in correlation with lymph node status.

Variable			**LN+**	**LN-**	**p-value**
Age	**Median (range)**		58 (33, 87)	60 (26, 90)	NS
Tumor size	**Median (range)**		1.8 (0, 10)	1 (0, 11)	<.0001
Gender	**F**		123 (98.4)	270 (99.6)	NS
	M		2 (1.6)	1 (0.4)	
Histology and grade*	**DCIS (46)**	**Grade 1**	0 (0)	1 (2.2)	NS
		Grade 2	0 (0)	23 (51.1)	
		Grade 3	1 (100)	21 (46.7)	
		Total	1	45	
	Ducal (294)	**Grade 1**	24 (22)	55 (29.7)	NS
		Grade 2	52 (47.7)	77 (41.6)	
		Grade 3	33 (30.3)	53 (28.7)	
		Total	109	185	
	Lobular (45)	**Grade 1**	4 (30.8)	12 (37.5)	NS
		Grade 2	9 (69.2)	19 (59.4)	
		Grade 3	0 (0)	1 (3.1)	
		Total	13	32	
	Other (11)	**Grade 1**	0 (0)	4 (44.4)	NS
		Grade 2	1 (50)	4 (44.4)	
		Grade 3	1 (50)	1 (11.1)	
		Total	2	9	
Multifocality	**Negative**		82 (65.6)	223 (82.3)	0.0005
	Positive		43 (34.4)	48 (17.7)	
T-stage	**1**		76 (61.8)	177 (80.1)	0.0008
	2		41 (33.3)	40 (18.1)	
	3		6 (4.9)	4 (1.8)	
ER and/or PR	**Negative**		18 (14.4)	42 (15.5)	NS
	Positive		107 (85.6)	229 (84.5)	

* 3 cases were microinvasive carcinoma (not included in the analysis).

Table 2. Frozen section versus touch preparation (patient-based) including all types of metastases (macro-, micro- and isolated tumor cells).

	Frozen section (n=73)	Touch preparation (n=323)	Total (n=396)	P value
True negative	26 (35.6)*	246 (76.2)	272 (68.7)	<0.0001
True positive	42 (57.5)	42 (13)	84 (21.2)	
False negative	5 (6.9)	35 (10.8)	40 (10.1)	
False positive	0 (0)	0 (0)	0 (0)	
Sensitivity	89.4	54.5	67.7	
Specificity	100	100	100	
Negative predictive value	83.9	87.5	87.2	
Positive predictive value	100	100	100	
Overall accuracy	93.2	89.2	89.9	

*Number (percentage)

Table 3. Frozen section versus touch preparation (node-based) including all types of metastases (macro-, micro-and isolated tumor cells).

	Frozen section (n=133)	Touch preparation (n=1137)	Total (n=1270)	P value
True negative	75 (56.4)*	1024 (90.1)	1099	<0.0001
True positive	48 (36.1)	56 (4.9)	104	
False negative	10 (7.5)	57 (5)	67	
False positive	0 (0)	0 (0)	0(0)	
Sensitivity	82.6	49.6	60.8	
Specificity	100	100	100	
Negative predictive value	88.2	94.7	94.3	
Positive predictive value	100	100	100	
Overall accuracy	92.5	95	94.7	

*Number (percentage)

Table 4. Frozen section versus touch preparation in detecting micrometastases and isolated tumor cells.

	Frozen section (n=85)	Touch preparation (n=1071)	Total (n=1156)	P value*
True negative	75 (88.2)**	1024 (95.6)	1099 (95)	<0.0001
True positive	5 (5.9)	6 (0.6)	11 (1)	
False negative	5 (5.9)	41 (3.8)	46 (4)	
False positive	0 (0)	0 (0)	0 (0)	
Sensitivity	50	12.8	19.3	
Specificity	100	100	100	
Negative predictive value	93.8	96.2	96	
Positive predictive value	100	100	100	
Overall accuracy	94.1	96.2	96	

*Fisher's exact test; **number (percentage)

The sensitivity of FS in detecting micrometastases has been reported to be between 10% and 61% with a median of 28%.[5] While using TP, the sensitivity ranged from 5% to 57% with a median of 22%.[6] Therefore, regardless of the method used (TP vs. FS), micrometastases is still problematic to detect intraoperatively. In our study, we found that FS detected 5 of 10 (50%) of SLNs with micrometastases, while TP detected 5 of 56 (19.3%).

Occult tumor metastases have been proposed to be in part responsible for tumor recurrence in node negative disease. [9-14] Moreover, in the National Surgical Adjuvant Breast and Bowel Project B-32 trial, SLNs were cut through the block until no tissue was left. The slides were reviewed and found that patients classified as "negative for occult metastases" had at least an 8.9% chance of having undetected ITCs and a 2.2% chance of having undetected micrometastases.[23] So there is a good chance that tumor metastases left undetected in a SLN-PS due to the phenomenon of "occult tumor". We intended to explore the possibility of occult tumor due to FS sectioning procedure. We found that although the metastases size correlation between FS and PS was high, there were 3 SLNs positive by FS but negative on PS and one SLN that was positive by TP but negative by FS and later on PS. This phenomenon could be due to three factors combined; first, small tumor volume; second, harsh tissue sectioning during FS and/or PS preparation; and third, metastases present close to the SLN surface (**Figure 2**). The amount of tissue wasted during FS preparation could reach up to 0.5-mm thick, particularly when the SLN is fatty and hard to cut. On the other hand, when FS was performed on SLNs with macrometastases, all positive SLNs remained positive on PS. One lymph node became a micrometastasis on PS after being macrometastases on FS.

Table 5. Cases with partial discrepancy between frozen section, touch preparation and permanent section with metastases size.

Case#	TP	FS	PS	Size-mm
1	Positive	Positive	Negative	0.5
2	NA	Positive	Negative	0.5
3	Positive	Positive	Negative	1
4	Positive	Negative	Negative	NA

If FS is routinely used, this procedure detects higher number of patients who are eligible for a simultaneous axillary lymph node dissection with the SLN biopsy procedure. This would spare the patient from another surgery with less overall cost. However, there is one caveat to this approach which is the possibility of LN understaging. Lymph node positivity could be the reason for a patient to receive chemotherapy. Therefore, lymph node understating would deny some patients an effective and may be curable chemotherapy. If TP is used instead, there will be a higher number of patients who will undergo a second surgery with higher overall cost. However, these patients would have less chance of understaging and ultimately higher survival.

Taking these possibilities together, we suggest doing FS on grossly suspicious SLNs only, as this approach would not understage patients. On the other hand, when a SLN is grossly negative, we think TP is the preferred approach. However, to prove the understaging phenomenon when FS procedure is used, a large prospective study examining the tissues sectioned from the SLN during the FS preparation should be performed.

CONFLICT OF INTEREST
None.

REFERENCES

1. Fitzgibbons PL, Page DL, Weaver D, et al. Prognostic factors in breast cancer: College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med. 2000;124(7):966-978.
2. Saez RA, McGuire WL, Clark GM. Prognostic factors in breast cancer. Semin Surg Oncol. 1989;5(2):102-110.
3. Leidenius MH, Krogerus LA, Toivonen TS, et al. The feasibility of intraoperative diagnosis of sentinel lymph node metastases in breast cancer. J Surg Oncol. 2003;84(2):68-73.
4. Rönkä R, Smitten K, Sintonen H, et al. The impact of sentinel node biopsy and axillary staging strategy on hospital costs. Ann Oncol. 2004;15(1):88-94.
5. Tille JC, Egger JF, Devillaz MC, et al. Frozen section in axillary sentinel lymph nodes for diagnosis of breast cancer micrometastasis. Anticancer Res. 2009;29(11):4711-4716.
6. Tew K, Irwig L, Matthews A, et al. Meta-analysis of sentinel node imprint cytology in breast cancer. Br J Surg. 2005;92(9):1068-1080.
7. Fisher B, Bauer M, Wickerham L, et al. Relation of number of positive axillary nodes to the prognosis of patients with primary breast cancer: an NSABP update. Cancer. 1983;52(9):1551-1557.
8. Rosen PP, Saigo PE, Braun DW Jr, et al. Predictors of recurrence in stage I (T1N0M0) breast carcinoma. Ann Surg. 1981;193(1):15-25.
9. Cummings MC, Walsh MD, Hohn BG, et al. Occult axillary lymph node metastases in breast cancer do matter: results of 10-year survival analysis. Am J Surg Pathol. 2002;26(10):1286-1295.
10. Kahn HJ, Hanna WM, Chapman JA, et al. Biological significance of occult micrometastases in histologically negative axillary lymph nodes in breast cancer patients using the recent American Joint Committee on Cancer breast cancer staging system. Breast J. 2006;12(4):294-301.
11. Neville AM, Price KN, Gelber RD, et al. Axillary node micrometastases and breast cancer. Lancet. 1991(8749);337:1110.
12. Trojani M, de Mascarel I, Bonichon F, et al. Micrometastases to axillary lymph nodes from carcinoma of breast: detection by immunohistochemistry and prognostic significance. Br J Cancer. 1987;55(3):303-306.
13. de Mascarel I, Bonichon F, Coindre JM, et al. Prognostic significance of breast cancer axillary lymph node micrometastases assessed by two special techniques: reevaluation with longer follow-up. Br J Cancer. 1992;66(3):523-527.
14. Cote RJ, Peterson HF, Chaiwun B, et al. Role of immunohistochemical detection of lymph-node metastases in management of breast cancer: International Breast Cancer Study Group. Lancet. 1999;354(9182):896-900.
15. Pickren JW. Significance of occult metastases: a study of breast cancer. Cancer. 1961;14:1266-1271.

16. Fisher ER, Swamidoss S, Lee CH, et al. Detection and significance of occult axillary node metastases in patients with invasive breast cancer. Cancer. 1978;42(4):2025-2031.
17. Wilkinson EJ, Hause LL, Hoffman RG, et al. Occult axillary lymph node metastases in invasive breast carcinoma: characteristics of the primary tumor and significance of the metastases. Pathol Annu. 1982;17(2):67-91.
18. Zhang PJ, Reisner RM, Nangia R, et al. Effectiveness of multiple- level sectioning in detecting axillary nodal micrometastasis in breast cancer: a retrospective study with immunohistochemical analysis. Arch Pathol Lab Med. 1998;122(8):687-690.
19. International (Ludwig) Breast Cancer Study Group. Prognostic importance of occult axillary lymph node micrometastases from breast cancers. Lancet. 1990;335(8705):1565-1568.
20. Hainsworth PJ, Tjandra JJ, Stillwell RG, et al. Detection and significance of occult metastases in node-negative breast cancer. Br J Surg. 1993;80(4):459-463.
21. Kane JM 3rd, Edge SB, Winston JS, et al. Intraoperative pathologic evaluation of a breast cancer sentinel lymph node biopsy as a determinant for synchronous axillary lymph node dissection. Ann Surg Oncol. 2001;8(4):361-367.
22. Edge, BS, editor. *AJCC cancer staging manual*. 7th ed. Part VII-breast. Springer: New York, USA; 2010. 345.
23. Weaver DL, Le UP, Dupuis SL, et al. Metastasis detection in sentinel lymph nodes: comparison of a limited widely spaced (NSABP protocol B-32) and a comprehensive narrowly spaced paraffin block sectioning strategy. Am J Surg Pathol. 2009;33(11):1583-1589.