Original Research

False-Negative Interpretation of Breast Sentinel Lymph Node Touch Preps: Analysis of the Causes with Suggestions to Improve Diagnostic Accuracy

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Sentinel lymph node (SLN) biopsy has become widely accepted as an important procedure in staging breast cancer. False-negative results of touch prep (TP) examination at time of SLN biopsy requires additional surgery, delaying treatment and increasing cost. Therefore, we have analyzed our experience with false-negative interpretation on SLN TP's. Eight-hundred and three consecutive SLN biopsies from 2003 to 2005 were obtained from the pathology archive of Roswell Park Cancer Institute. The intraoperative consultation results were correlated with the final diagnoses. Twenty-five SLN intraoperative consultations had false-negative TP's [false-negative rate = 3.1% (25/803), including 9 metastatic lobular carcinomas and 16 metastatic ductal carcinomas]. These cases were re-evaluated by 3 pathologists independently, and the metastases in the SLN sections were confirmed by positive cytokeratin staining. Size of the metastatic focus, nuclear grade and the adequacy of TP's were analyzed with regard to the cause of false-negative results. On re-screening of TP's, we found that rare tumor cells of low nuclear grade were identified on 28% (7/25) of the TP's (3 metastatic lobular carcinomas and 4 metastatic ductal carcinomas). In the remaining 72% (18/25) of TP's, re-screening revealed no evidence of tumor. Evaluation of these TP's demonstrated that 50% (9/18) were unsatisfactory for evaluation or limited by scant cellularity. While cases that remained negative on re-screening tended to have smaller measured foci of tumor in the SLN (Average 0.65 mm vs. 0.94 mm from cases that were positive on re-screening), there was considerable overlap between these two groups. In conclusion, TP's with scant cellularity, unsatisfactory TP's and failure to identify tumor cells with low nuclear grade were found to significantly contribute to false-negative interpretations. We suggest that an additional TP or frozen section may be necessary if the cellularity of the initial TP is limited. Correlation with the original core biopsy may be of value to help in identifying cancer cells of low nuclear grade.

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INTRODUCTION

A sentinel lymph node (SLN) is the first lymph node to receive afferent lymphatic drainage from the primary tumor. SLN biopsy examination is the current modality for evaluating the axilla in breast cancer patients.[1-4] Numerous studies have demonstrated that SLN biopsy can determine axillary nodal status for breast cancer, predicting the risk of additional nodal metastases.[1,3-6] This procedure not only allows the surgeon to make an individualized decision regarding the need for completion axillary lymph node dissection, but also permits it to be performed during the same mastectomy procedure if metastatic tumor is found.[2,7] However, intraoperative diagnostic techniques such as touch prep examination, often carry the risk of false-negative results.[8] In this study, we have evaluated our experience with false-negative interpretations on cytologic examination of sentinel lymph nodes, analyzed the possible causes and provided suggestions to improve the diagnostic accuracy.

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METHODS

Pathology reports from 803 consecutive SLN biopsies from 2003 to 2005 were obtained from the pathology archive of Roswell Park Cancer Institute. In all of these cases, during intraoperative consultation, the SLN's were serially sectioned perpendicular to the long axis and touch preps were derived from the exposed cut surfaces. Then, the SLN's were formalin-fixed for permanent sections. In this study, these intraoperative consultation results were re-evaluated retrospectively and correlated with the final diagnoses by three pathologists independently. The metastases in the SLN sections were confirmed by cytokeratin staining. Size of the metastatic focus, nuclear grade and the adequacy of TP's were analyzed regarding the cause of false-negative results.

RESULTS

We found that 25 out of 803 SLN intraoperative consultations had false-negative interpretations, including 9 metastatic lobular carcinoma cases and 16 metastatic ductal carcinoma cases **(Table 1)**. The false-negative rate is 3.1% (25/803). On re-screening, rare tumor cells of low nuclear grade were identified on 28% (7/25) of TP's, including 3 metastatic lobular carcinoma cases and 4 metastatic ductal carcinoma cases. Examples of metastatic ductal carcinoma on TP and in SLN are shown in **Figure 1** and **Figure 2,** respectively. In the remaining 72% (18/25) of TP's, re-screening revealed no evidence of metastatic tumor. Evaluation of these TP's demonstrated that 50% (9/18) were unsatisfactory for evaluation or limited by scant cellularity. While cases that remained negative on re-screening tended to have smaller measured foci of tumor in the SLN (Average 0.65 mm vs. 0.94 mm from cases that were positive on re-screening), there was considerable overlap between these two groups.

Table 1. Axillary SLN with False Negative Interpretation between 01/2003-06/2005.

TP	TP Original Dx	TP Rescreen by A	TP Rescreen by B	TP Rescreen by C	Lymph Node Dx	Nuclear Grade	Primary Tumor Dx	Causes for error
1	Neg	Neg	Neg	Neg	Micro mets (1.0 mm)	II	Ductal CA	Limited by SC* + DA**
2	Neg	Neg	Neg	Neg	Micro mets (0.5 mm)	I	Ductal CA	SAT#
3	Neg	Neg	Neg	Neg	Micro mets (1.1 mm)	I	Lobular CA	Limited by SC*
4	Neg	Neg	Neg	Neg	Macro mets (3.0 mm)	I	Ductal CA	Limited by SC* + DA**
5	Neg	Neg	Neg	Neg	Micro mets (2.0 mm)	I	Lobular CA	Limited by TS***
6	Neg	Neg	Neg	Neg	Submicro mets (0.1 mm)	II	Lobular CA	Limited by SC*
7	Neg	Neg	Neg	Neg	Micro mets (1.5 mm)	I	Ductal CA	SAT#
8	Neg	Neg	Neg	Neg	Micro mets (0.7 mm)	II	Ductal CA	Limited by SC*
9	Neg	Neg	Neg	Neg	Micro mets (1.0 mm)	II	Ductal CA	Limited by SC*
10	Neg	Neg	Neg	Neg	Micro mets (0.3 mm)	II	Ductal CA	SAT#
11	Neg	Neg	Neg	Neg	Macro mets (3 mm)	II	Ductal CA	Limited by TS***
12	Neg	Neg	Neg	Neg	Submicro mets (0.1 mm)	II	Ductal CA	UNSAT##
13	Neg	Neg	Neg	Neg	Micro mets (0.3 mm)	II	Ductal CA	SAT#
14	Neg	Neg	Neg	Neg	Submicro mets (0.1 mm)	II	Ductal CA	SAT#
15	Neg	Neg	Neg	Neg	Micro mets (0.7 mm)	I	Lobular CA	UNSAT##
16	Neg	Neg	Neg	Neg	Micro mets (2.0 mm)	I	Lobular CA	Limited by SC*+ DA**
17	Neg	Neg	Neg	Neg	Micro mets (1.0 mm)	I	Mixed Ductal/Lobular CA	SAT#
18	Neg	Neg	Neg	Neg	Micro mets (0.2 mm)	I	Mixed Ductal/Lobular CA	SAT#
19	Neg	**Pos**	**Pos**	**Suspicious**	Macro mets (2.5 mm)	I	Tubulolobular CA	SAT#
20	Neg	**Pos**	**Pos**	**Susp-prob Pos**	Micro mets (1.5 mm)	II	Ductal CA	Limited by TS***
21	Neg	**Pos**	**Pos**	**prob pos**	Micro mets (0.7 mm)	II	Ductal CA	Limited by TS***
22	Neg	**Pos**	**Pos**	**Pos**	Submicro mets (< 1 mm)	I	Lobular CA	SAT#
23	Neg	**Pos**	**Pos**	**Pos**	Micro mets (0.9 mm)	I	Lobular CA	SAT#
24	Neg	**Pos**	**Pos**	**Pos**	Micro mets (0.3 mm)	II	Ductal CA	SAT#
25	Neg	**Pos**	**Pos**	**Pos**	Micro mets (0.5 mm)	I	Ductal CA	SAT#

*SC: Scant cellularity; **DA: Dry artifact; ***TS: Thick smear; #SAT: Satisfactory; ##UNSAT: Unsatisfactory.

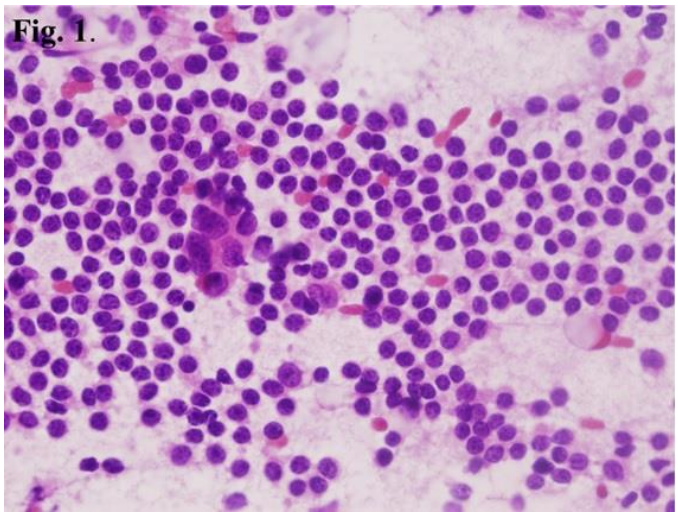

Figure 1. Metastatic ductal carcinoma on touch prep.

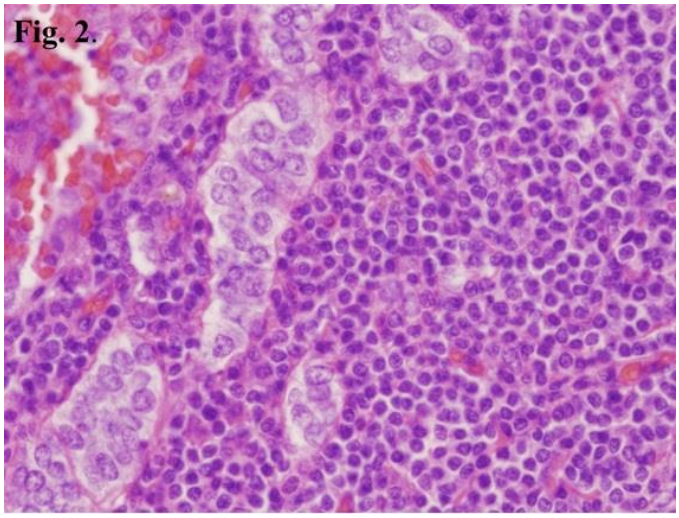

Figure 2. Metastatic ductal carcinoma in SLN.

DISCUSSION

SLN biopsy is commonly used in the evaluation of breast cancer patients. Axillary lymph node status is considered the most significant prognostic factor for breast cancer outcome, and treatment decisions are based on the presence or absence of nodal disease.[1,2] According to the revised American Joint Committee on Cancer (AJCC) staging: SLN metastases were classified as follows;[9] (1) immunohistochemistry positive if only single keratin-positive cells or clusters were present and were not observed with standard tissue stains; (2) submicrometastatic if tumors were less than 0.2 mm (excluding IHC positive); (3) micrometastatic if tumors were larger than 0.2 mm but </=2 mm, or (4) macrometastatic if tumors were larger than 2 mm. A previous study has found a significantly poorer prognosis associated even with metastases less than 2 mm in size (micro- and submicrometastasis), suggesting that such small metastases cannot be safely overlooked.[10] In addition, Kamath et al showed that sentinel lymph node micrometastases, regardless of identification techniques, inferred a risk of 15.2% for non sentinel lymph node (NSLN) involvement. As the volume of tumor in the SLN increased, the risk of NSLN metastases also increased.[5]

Touch prep is often used for intraoperative examination of SLN's in breast cancer. This allows axillary lymph node dissection to be performed immediately for tumor-positive nodes when mastectomy is the surgery of choice.[2,7] However, it has a high false-negative rate, particularly in patients with micrometastases.[5] In 2006, Puqliese et al reported that the chances of false-negative intraoperative consultation increased with decreasing size of the metastasis.[6] We observed similar correlation between the size of metastatic tumor and false-negativity. However, due to small sample size, the correlation is not significant. We predict that future studies with larger numbers of cases should verify the above observation.

Different methods have been tried to reduce the false negativity rate of breast SLN biopsy. Cytokeratin immunohistochemical staining of the breast SLN detects micrometastatic disease, which is frequently missed on routine H&E stain, providing more accurate staging of the regional lymph nodes in patients with breast cancer.[11] However, the role of rapid immunohistochemistry for cytokeratin during intraoperative consultation is controversial. Johnston et al reported that rapid immunohistochemistry for cytokeratin is a more sensitive method for detecting breast cancer metastases in SLN's than TP's and frozen sections.[12] In contrast, Beach et al showed that the method of rapid immunohistochemistry to detect metastasis was the least sensitive when compared with TP's, frozen sections, and permanent sections.[13] Further, Celebrioglu et al divided the metastases into micrometastases and macrometastases, and found that the sensitivity for detection of micrometastases was not substantially increased by the use of intraoperative immunohistochemistry.[14] Molecular techniques such as polymerase chain reaction (PCR) offer even more sensitive methods for detecting occult metastasis in SLN's. However, it remains as a research tool due to its high false positive rate.[10]

In this study, we have found that scant cellularity, technical limitations (i.e. too thick, air drying) of TP's and failure to identify tumor cells with low nuclear grade significantly contribute to false-negative interpretations. We suggest that an additional TP or frozen section may be necessary if the cellularity of the initial TP is unsatisfactory or if there are correctable technical limitations on the initial TP. Correlation with the original core biopsy may be of value to help identify cancer cells of low nuclear grade.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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