Original Article

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Accuracy of Intraoperative Sentinel Lymph Node Evaluation by Imprint Cytology in Breast Cancer: A 12-Year Single Center Experience With 2,528 Patients

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ABSTRACT

Objective: Sentinel lymph node biopsy (SLNB) is a key procedure for evaluating axillary lymph node status in early breast cancer, offering lower morbidity than axillary lymph node dissection. Intraoperative evaluation (IOE) of sentinel lymph nodes (SLNs) with methods like frozen section (FS) and imprint cytology (IC) aid in making immediate surgical decisions, although IOE accuracy may vary due to several factors.

Materials and Methods: This retrospective study involved 2,528 patients with invasive breast cancer who underwent SLNB at a single institution from 2012 to 2024. Primarily, IC was used for intraoperative assessment, while FS was selectively performed in certain cases, such as with suspicious macroscopic findings or after neoadjuvant chemotherapy (NAC). The final diagnosis relied on permanent sections with serial step-leveling and classification of metastasis size.

Results: IOE showed a sensitivity of 65.8% and specificity of 97% for detecting lymph node metastases. The combination of IC and FS yielded higher sensitivity (76.1%) compared to IC alone (64.1%), particularly for isolated tumor cells (ITC). Patients treated with NAC exhibited slightly lower IOE accuracy (83.8%) compared to those without NAC (85.9%). False negatives were more common in cases of micrometastasis, ITC, and invasive lobular subtype. Excluding micrometastasis and ITC significantly enhanced IOE accuracy.

Conclusion: The accuracy of intraoperative SLN evaluation is affected by size of the metastasis, tumor subtype, and prior NAC. While IC is acceptable for IOE, combining IC and FS is advised, especially in the setting of earlier NAC, to enhance accuracy for small metastatic foci.

Keywords: Breast cancer; intraoperative evaluation; neoadjuvant chemotherapy; sentinel lymph nodes

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Key Points

- Parameters such as the extent of metastasis, tumor subtype, and the use of neoadjuvant chemotherapy (NAC) in the intraoperative evaluation (IOE) of sentinel lymph nodes (SLNs) affect the accuracy of the results.
- Imprint cytology (IC) is considered an acceptable method for the IOE of SLNs in a primary surgery setting.
- However, IC and frozen section are both recommended in the setting of a patient having received NAC.

Introduction

Sentinel lymph node biopsy (SLNB) has been introduced as an effective and safe procedure to assess axillary lymph node status in patients with early breast cancer and clinically negative lymph nodes. SLNB with no further axillary lymph node dissection has been shown to reduce postoperative long-term morbidity without compromising local disease control (1-5).

The two most common methods used for intraoperative evaluation (IOE) of the sentinel lymph node include frozen section (FS) and imprint cytology (IC) (6-10). In primary surgery, detecting lymph node metastasis intraoperatively by IC alone has an estimated sensitivity of 63%, ranging from 34% to 95%. In contrast, the sensitivity for FS is 86% although this also varies widely from 44% to 99.8% (6, 11). Like any other assay, multiple factors influence the accuracy of results in

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the IOE of sentinel lymph nodes. Some factors include the evaluation method, quality of the IC or FS slides, the size of the metastasis, tumor type, whether the patient received treatment before surgery, and the experience of the pathologists interpreting the slides (12, 13).

The aim of the present study was to assess the accuracy of IC and FS in the IOE of sentinel lymph nodes and investigate factors that contribute to low accuracy.

Materials and Methods

Patient Selection

A total of 2,528 patients with invasive breast cancer who underwent SLNB were selected through a database search at the Pathology Department of İstanbul Medical Faculty from 2012 to 2024. Clinicopathologic parameters, including patient age, histologic tumor type, pathologic tumor stage, size of nodal metastasis, and pathologic nodal stage, were obtained from patients records.

Intraoperative Evaluation

At our institution, the pathology department has a subspecialty practice model. However, IOEs are performed by pathologists from all subspecialties, including those in the breast subspecialty. Our standard intraoperative lymph node assessment procedure was used in all cases included in this study. Lymph nodes were serially sectioned into 2- to 3- 3-mm-thick cross sections; ICs were performed by imprinting the whole cut surface on one side of all cross sections, with at least two IC slides performed on most of the lymph nodes; and tissue scraping of grossly suspicious areas was performed in some cases. Although the FS was not a part of the standard IOE procedure in our institution, in some situations, such as suspicious macroscopic findings, inadequate touch imprint preparation, and especially when the patient had undergone neoadjuvant chemotherapy (NAC), preferred to perform FSs in addition to ICs. IC and FS slides were stained with routine hematoxylin-eosin (H&E) stain and interpreted by a single pathologist assigned for FS service.

Microscopic Evaluation

For the final diagnosis, H&E-stained, 3-step levels of the entire lymph node were assessed. An unstained level between the two H&E slides was retained for possible cytokeratin staining. The size of nodal metastasis was classified according to the eighth edition of the American Joint Committee on Cancer Staging System. Nodal metastases were classified as isolated tumor cells (ITC) (<0.2 mm), micrometastasis (>0.2 mm to <2 mm), or macrometastasis (>2 mm) (14).

Statistical Analysis

All data were analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 21.0 (IBM Corp, Armonk, NY, USA). The χ^2 and Fisher exact tests were applied for categorical variables, and the Mann-Whitney U test was used for continuous variables. Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) respectively, and overall accuracy of IOE for detecting axillary lymph node metastases were calculated. A $p<0.05$ was considered significant in all comparisons. The lymph nodes with an intraoperative diagnosis of "atypical" were excluded from the statistical accuracy analysis.

Results

The median (range) age of the 2,528 patients with invasive breast cancer who underwent SLNB was 52 (23–90) years. A total of 7,204 lymph nodes were identified from the 2,528 cases, with a median number of lymph nodes of 2.85 (1–14) lymph nodes. Among the study group, 1,757 (69.5%) cases were invasive ductal carcinoma, 164 (6.5%) cases were invasive lobular carcinoma, and 607 (24%) cases were another histological subtype. Of the patients, 645 (25.5%) were treated with NAC. The T stage of the cases (T1, T2, T3, T4) were 1,018 (40.3%), 1,302 (51.5%), 178 (7%), and 30 (1.2%), respectively. Of the patients with intraoperative pathologic evaluation, 2,238 (88.5%) were evaluated with IC, and 290 (11.5%) were assessed with IC and FS. Of the 2,528 cases that underwent IOE, 2,161 (85.5%) were evaluated by non-breast pathologists, and 367 (14.5%) were assessed by breast pathologists.

Of the 2,528 cases examined in this study, 1,713 (67.8%) were interpreted as negative and 650 (25.7) as positive. In 165 (6.5%) patients, the IOE was reported as 'atypical' rather than benign or malignant. In the final diagnosis, 1,052 (41.6%) of 2,528 cases were positive for any tumor cells. Of the metastatic cases, 773 (73.5%) contained macrometastasis, 183 (17.4%) micrometastasis, and 96 (9.1%) had ITC. The overall sensitivity, specificity, PPV, and NPV for identifying lymph node metastases was 65.8%, 99%, 97.9%, and 80.7%, respectively. When micrometastasis and ITC were excluded from the analysis, sensitivity, specificity, PPV, and NPV of IOE for identifying lymph node metastases were 81.6%, 99%, 97.7%, and 91.3%, respectively.

The sensitivity, specificity, PPV, and NPV rates of sentinel lymph nodes evaluated by IC alone were 64.1%, 99%, 97.5%, and 80.8%, respectively. In 290 (11.5%) patients evaluated by IC and FS, the sensitivity, specificity, PPV, and NPV were 76.1%, 100%, and 79.8%, respectively. In the IOEs performed only with IC, the correlation rates for macrometastasis, micrometastasis, and ITC were 80.7%, 24.1%, and 11.1%, respectively. In the examination performed with IC and FS, the correlation rates were 86.2%, 29.4%, and 37.5%, respectively. In the IOE, the use of the FS method was significantly different only when detecting ITC (p = 0.037). The relationships of clinicopathological parameters with IOE and final diagnostic agreement are shown in Table 1. The distribution of cases according to the IOE method is shown in Figure 1.

IOE had a sensitivity of 67.3%, a specificity of 99%, and an accuracy of 83.8% in patients treated with NAC. In patients without NAC, IOE had a sensitivity of 65.2%, a specificity of 99%, and an accuracy of 85.9% (p = 0.206). The distribution of cases according to NAC status is shown in Figure 2.

The false-negative rate was 13.1% for IOE. Among 331 false-negative results, macrometastasis, micrometastasis, and ITC were identified in 39.9%, 36.8%, and 23.3%, respectively. In sentinel lymph nodes with false negative results, micrometastasis and ITC, and invasive lobular subtype were observed at higher frequencies than in the whole cohort ($p<0.001$).

Of the 650 sentinel lymph nodes evaluated as positive in IOE, 14 were false-positive. All these patients were evaluated with IC by a non-

Table 1. Comparison of pathologic and clinical parameters between concordant and discordant cases in intraoperative evaluation

	All patients** (*n* = 2,363) (%)	Concordant result (true pos+true neg) (*n* = 2,018) (%)	Discordant result (false neg+false pos) (*n* = 345) (%)	*p*
Age (mean)	52.01	52.18	51.01	0.077
Histologic subtype				
Ductal	1,651 (69.9)	1,404 (85)	247 (15)	
Lobular	145 (6.1)	112 (77.2)	33 (22.8)	**0.002**
Other	567 (24)	502 (88.5)	65 (11.5)	
Neoadjuvant chemotherapy				
No	1,803 (76.3)	1,549 (85.9)	254 (14.1)	0.206
Yes	560 (23.7)	469 (83.8)	91 (16.3)	
T stage				
T1	963 (40.8)	832 (86.4)	131 (13.6)	
T2	1,212 (51.3)	1,023 (84.4)	189 (15.6)	0.374
T3	161 (6.8)	140 (86.9)	21 (13.1)	
T4	27 (1.1)	23 (85.2)	4 (14.8)	
N stage				
N0	1,443 (61.1)	1,361 (94.3)	82 (5.7)	
N1	690 (29.2)	443 (64.2)	247 (35.8)	**<0.001**
N2	175 (7.4)	161 (92)	14 (8)	
N3	55 (2.3)	53 (96.4)	2 (3.6)	
IOE methods				
IC	2,103 (89)	1,790 (85.1)	313 (14.9)	0.267
IC+FS	260 (11)	228 (87.7)	32 (12.3)	
Type of metastasis				
Macrometastasis	716 (30.4)	584 (81.6)	132 (18.4)	
Micrometastasis	162 (6.8)	40 (24.7)	122 (75.3)	**<0.001**
ITC	89 (3.8)	12 (13.5)	77 (86.5)	
Evaluating pathologist				
Breast pathologist	346 (14.6)	298 (86.1)	48 (13.9)	0.678
Non-breast pathologist	2,017 (85.4)	1,720 (85.3)	297 (14.7)	

IOE: Intraoperative evaluation; IC: Imprint cytology; FS: Frozen section; ITC: Isolated tumor cells;
** Intraoperative diagnosis of "atypical" (*n* = 165) were excluded from the statistical analysis

breast pathologist. A review of false-positive lymph node IC revealed reactive changes, including histiocytes and multinucleated giant cells from a prior biopsy site or regression site associated with NAC. Finally, of the 165 (6.5%) sentinel lymph nodes interpreted as atypical, 80 (48.5%) were interpreted as negative in permanent sections. Of these 165 patients, 85 (51.5%) were treated with NAC, and 135 (81.8%) were evaluated with IC.

Discussion and Conclusion

Intraoperative sentinel lymph node assessment to detect metastatic breast carcinoma has become the standard of care not only to avoid unnecessary axillary lymph node dissection but also to eliminate reoperation for completion of axillary lymph node dissection (14).

In previous studies, the overall sensitivity and specificity of IOE for the identification of sentinel lymph node metastases were reported to be 40–86% and 97–100%, respectively (15-19). In the present study, the overall sensitivity and specificity of IOE for identifying sentinel lymph node metastases were 65.8% and 97%, respectively.

Despite the variable accuracy, many pathologists, particularly those familiar with cytology preparations, prefer IC as it is technically easier to perform and offers a faster turnaround time. Another reason IC may be favored over FS is because it is technically challenging to cut fatty lymph nodes, and FS can deplete tissue and, therefore, possibly miss smaller metastasis. In a previous study (14), the sensitivity of IC was 37.5%, specificity was 100%, and NPV was 90.2%. In the literature,

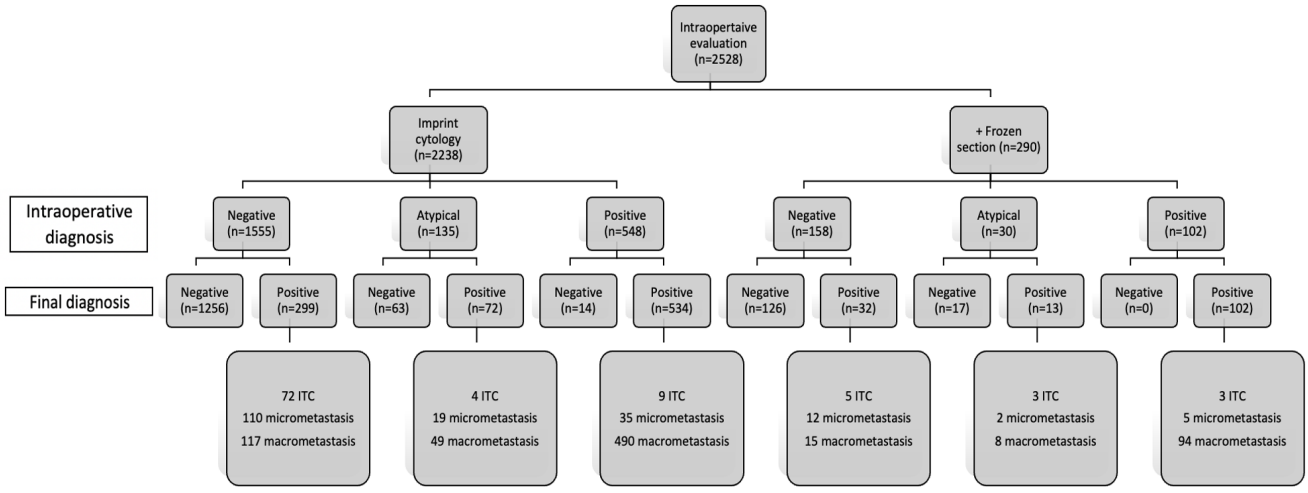

Figure 1. The distribution of cases according to the intraoperative evaluation method

ITC: Isolated tumor cells

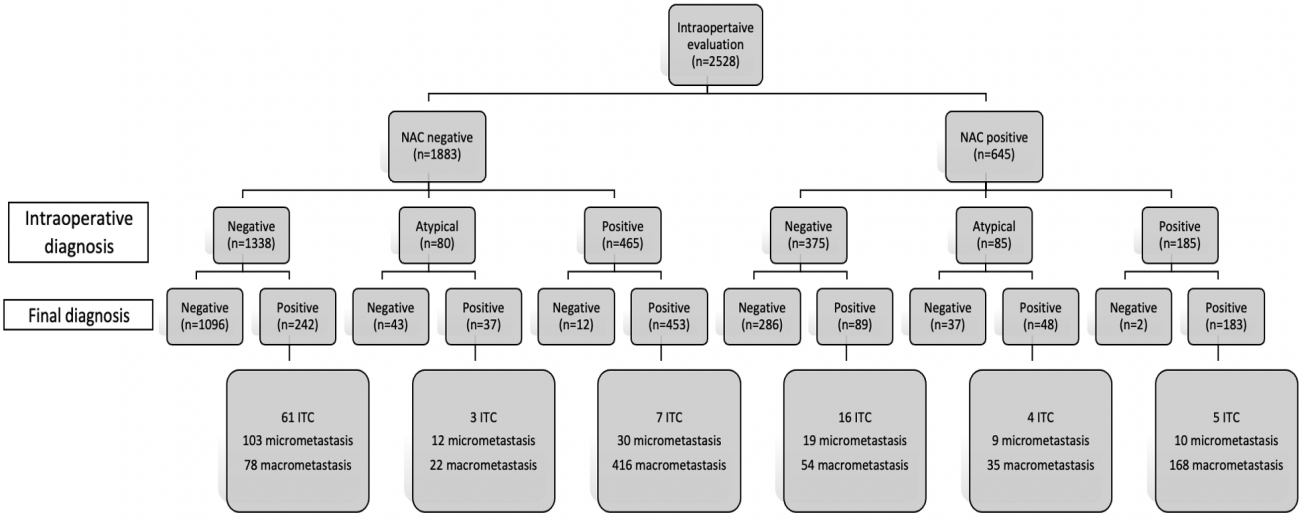

Figure 2. The distribution of cases according to neoadjuvant chemotherapy status

ITC: Isolated tumor cells

the reported sensitivity of IOE using IC varies between 34% and 95% (6, 15-19). In another study, IC alone had a sensitivity of 66.7% and specificity of 100%; FS alone had a sensitivity and specificity of 100%; and combined IC and FS had a sensitivity and specificity of 100% and 96%, respectively (20). In the present study, IC alone had a sensitivity of 64.1% and specificity of 99%; combined IC and FS had a sensitivity and specificity of 76.1% and 100%, respectively.

IOE of sentinel lymph node metastasis in patients treated with NAC can be challenging due to therapy effects. It has been suggested that the quality of IC preparations may decrease due to low cellularity and fibrosis after neoadjuvant treatment (21). In a study conducted after NAC, the sensitivity of IC was 61.8%, specificity and PPV were 100%, NPV was 82.4%, and accuracy was 86.3% (22). The sensitivity of IOE in the NAC setting in our study is within the range (38.6% to 87.9 %) reported by other studies (23-27). Our analysis revealed a lower accuracy rate for IOE of sentinel lymph nodes in patients treated with NAC (83.8%) compared with patients who had not received NAC (85.9%), even though the specificity was high in both settings.

According to clinical guidelines, patients with limited sentinel lymph node involvement may not require completion axillary lymph node dissection. Therefore, the ability to detect tumor deposits smaller than 2 mm must be balanced with its clinical benefit (28-30). The accuracy of IOE detecting metastases irrespective of the prior treatment status is much higher when micrometastasis and ITC are excluded from the analysis (23, 30). Our study also showed that IOE has a much higher

accuracy in detecting metastases when micrometastasis and ITC are excluded from the analysis.

Metastasis of invasive lobular carcinoma is known to have high false negative rates. Two previous studies have demonstrated high false negative rates for invasive lobular carcinoma for IOE of sentinel lymph node (14, 23). In general, the IOE of sentinel lymph nodes in invasive lobular carcinoma cases has lower sensitivity and accuracy than invasive ductal carcinomas in the present study.

The major strength of this study was that it included a large cohort of patients treated over a long period by varying grades of breast surgeons, and patient demographics were consistent with the breast cancer population. The limitations of our study were that it was non-randomized and retrospective.

In conclusion, parameters such as the size of the metastasis, tumor subtype, and presence of NAC in the IOE of sentinel lymph nodes affect the accuracy of the results. IC is considered an acceptable method for the IOE of sentinel lymph nodes in the primary surgery setting, while IC and FS are both recommended in the NAC setting.

Ethics

Ethics Committee Approval: Not necessary.

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: B.M., M.T., H.K.; Concept: A.B., S.O., E.Y.; Design: A.B., S.O., E.Y.; Data Collection or Processing: A.B., C.S.K., E.S; Analysis or Interpretation: A.B., S.B.; Literature Search: A.B.; Writing: A.B., S.O., E.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

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