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Intraoperative Assessment of Sentinel Lymph Nodes in Breast Cancer Patients Post-Neoadjuvant Therapy

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Abstract

Background: Shift toward minimizing axillary lymph node dissection in patients with breast cancer post neoadjuvant therapy has led to the assessment of sentinel lymph nodes by frozen section intraoperatively to determine the need for axillary lymph node dissection. However, few studies have examined the accuracy of sentinel lymph node frozen section after neoadjuvant therapy. Our objective is to compare the accuracy of sentinel lymph node frozen section in patients with breast cancer with and without neoadjuvant therapy and to identify features that may influence accuracy. **Design:** We identified 161 sentinel lymph node frozen section from 77 neoadjuvant therapy patients and 255 sentinel lymph node frozen section from 88 non-neoadjuvant therapy patients diagnosed between 2010 and 2016 in 2 institutions. The frozen section diagnoses were compared to the final diagnoses, and clinicopathologic data were analyzed. **Results:** The sensitivity, specificity, and accuracy of frozen section analysis were comparable between neoadjuvant therapy patients and non-neoadjuvant therapy patients (71.9% vs 50%, 100% vs 100%, and 88.3% vs 81.8%). Nine (11.7%) of 77 neoadjuvant therapy patients had discordant results, most often due to undersampling (tumor absent on frozen section slide). Four of these patients subsequently underwent axillary lymph node dissection. Discordant results (all false negatives) were significantly more likely in neoadjuvant therapy patients with Estrogen Receptor-positive/HER2-negative status, and in sentinel lymph node with pN1mic and pN0i+ deposits; age, preneoadjuvant therapy lymph node status, histotype, nuclear grade, tumor size, and response to neoadjuvant therapy showed no significant differences. For non-neoadjuvant therapy cases, large tumor size, lobular histotype, and sentinel lymph node with pN1mic and pN0i+ were associated with false-negative frozen section assessment. **Conclusion:** Sentinel lymph node frozen section diagnosis post-neoadjuvant therapy has comparable sensitivity, specificity, and accuracy to the sentinel lymph node frozen section diagnosis in the non-neoadjuvant therapy setting.

Keywords

neoadjuvant therapy, breast carcinoma, sentinel lymph node biopsy, intraoperative assessment

Abbreviations

ALND, axillary lymph node dissection; BC, breast cancer; FNR, false-negative rates; FS, frozen section; H&E, hematoxylin and eosin; ITC, isolated tumor cells; NAT, neoadjuvant therapy; SLN, sentinel lymph node. ER, Estrogen Receptor.

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Introduction

Sentinel lymph node (SLN) biopsy has become the gold standard for staging of axillary lymph node status in patients with breast cancer (BC). It is recognized that SLN biopsy can predict axillary lymph nodes status accurately.[1-4] In the neoadjuvant therapy (NAT) setting, intraoperative SLN assessment is less established but is increasingly being utilized in favor of

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axillary lymph node dissection (ALND).[5] Intraoperative assessment of SLN biopsy in patients with BC post-NAT is beneficial because a positive finding results in an immediate axillary node dissection and avoids a separate subsequent completion ALND. As well, the post-NAT lymph node has the potential to have complete response to therapy, be downstaged, and subsequently patients with positive axillary lymph node pre-NAT may be spared from the morbidity of ALND. However, frozen section (FS) SLN post-NAT may demonstrate tumor bed changes and may have scant or focal residual carcinoma, making intraoperative analysis challenging.[6]

A few studies have examined the accuracy of FS SLN analysis post-NAT, with false-negative rates (FNRs) ranging from 20% to 26.2%.[7-10] These studies, however, lacked detailed analysis of pathological, radiological, and clinical parameters that may predict discordance between intraoperative assessment and final diagnosis. We studied the accuracy of intraoperative assessment of SLN biopsies after NAT in 2 institutions. Further, we identified clinical, radiological, and pathological parameters that may predict an increased risk of false-negative SLN intraoperative assessment.

Materials and Methods

Approval for this study was obtained from the ethics committees of the participating institutions. The data used for these analyses were collected between January 1, 2010, and December 31, 2015, for Sunnybrook Health Sciences Center and between March 2015 and November 2016 for North York General Hospital.

Eligibility Criteria

We included patients (1) with biopsy confirmed primary invasive BC, (2) had completed neoadjuvant chemotherapy (the regimen was at the discretion of the medical oncologist), (3) continued on to have primary resection of tumor and axillary lymph node sampling, and (4) with slides from the intraoperative and permanent specimens available for review. In total, 49 NAT patients from Sunnybrook Health Sciences Center, a tertiary cancer center, and 28 NAT patients from North York General Hospital, a community hospital, were studied. This was compared to 88 patients with BC from the non-NAT setting treated at Sunnybrook Health Sciences Center.

Sentinel Lymph Node Evaluation

Sentinel lymph node surgery incorporated injection of tracer to determine lymphatic drainage pathway. A combination of radiolabeled colloid and blue dye was used as tracer. The first lymph node(s) along the drainage pathway was identified by the tracer and the SLN biopsied. Intraoperatively, each SLN was sectioned into 2- to 3-mm thick cross-sections and submitted in toto. Each SLN was examined with at least 1 section stained with hematoxylin and eosin (H&E), and, if necessary, additional H&E sections were performed. After the intraoperative consultation, the SLN specimen was resubmitted in toto for permanent sections after formalin fixation. At least 1 H&E stained permanent section and one permanent section stained with CK8/18 immunostain were examined for each SLN. Positive SLNs were defined as those with any metastatic cells including isolated tumor cells (ITCs).

Table 1. Diagnostic Parameters of Intraoperative Analysis of Sentinel Lymph Nodes.

	Sensitivity	Specificity	PPV	NPV	Accuracy	FNR
NAT patients						
ITC included	71.9	100	100	83.3	88.3	28.1
ITC not included	79.3	100	100	88.2	91.9	20.7
Non-NAT patients						
ITC included	50	100	100	77.8	81.8	50
ITC not included	66.7	100	100	87.5	90	33.3

Abbreviations: FNR, false-negative rate; ITC, isolated tumor cell; NAT, neoadjuvant; NPV, negative predictive value; PPV, positive predictive value.

Analysis

Clinical, radiological, and pathological features were obtained from the electronic patient record and pathology database for all patients in the NAT and non-NAT setting. Univariate analysis was performed using 2-tailed Student *t* test to identify statistically significant differences between means, and Fisher exact test was used for categorical variables. *P* values of $<.05$ were considered statistically significant. All slides of cases with discordance between FS and permanent sections were retrieved in order to histologically evaluate the nature of the discrepancy.

Results

All discordant cases in non-NAT and NAT patients were false negatives, and there were no false positives. Combining results from both institutions, SLNs were correctly assessed with FS in 68 of 77 NAT patients. Sensitivity, specificity, and accuracy were 71.9%, 100%, and 88.3%, respectively (Table 1). False-negative rate in NAT patients was 28.1%. The cancer center and community hospital were similar in terms of sensitivity, specificity, and accuracy. Sensitivity, specificity, and accuracy were 68.8%, 100%, and 89.8%, respectively, at the cancer center and 75%, 100%, and 85.7% at the community hospital. In the non-NAT patients from the cancer center, 72 of 88 metastatic cases were identified correctly and sensitivity, specificity, and accuracy were 50%, 100%, and 81.8%, respectively. Finally, FNR in non-NAT patients was 50%.

Clinical, radiological, and pathological features of the total population of both NAT and non-NAT patient groups are presented in Table 2. In univariate analysis of clinicopathological features in NAT patients, an Estrogen Receptor (ER)-positive and HER2-negative breast biomarker profile and SLN metastasis that were ITCs or micrometastasis were identified as risk factors associated with discordant results between

Table 2. Patient and Tumor Characteristics.

	NAT Patients			Non-NAT Patients		
	Concordant Cases	Discordant Cases	*P*	Concordant Cases	Discordant Cases	*P*
Number of patients	68	9		72	16	
Average age at diagnosis (years)	50.5	48.3	.961	58	61	.380
Average number of SLN on FS	3.264	4.11	.119	3.1	3.4	.302
Lymph node cytology						
Positive	11	0	.258	0	0	1.000
Negative	14	3		14	4	
Not done	43	6		57	14	
Radiologic tumor size, mm						
≤20	14	1	.230	46	5	.027
>20 to ≤50	40	4		15	8	
>50	11	4		9	2	
Not available	3	0		2	1	
Histologic type						
IDC	61	7	.374	61	11	.022
ILC	2	0		5	5	
Other	5	2		6	0	
Type of LN metastasis						
No metastasis	45	0	.004	56	0	<.001
ITC	0	3		0	8	
Micromet	3	2		2	6	
Macromet	20	4		14	2	
Biomarker						
ER(+), HER2(+)	13	0	.022	8	3	.404
ER(+), HER2(−)	21	8		50	12	
ER(−), HER2(+)	5	0		4	0	
ER(−), HER2(−)	28	1		8	0	
ER(+), HER2(equiv)	0	0		1	1	
ER(−), HER2(equiv)	0	0		1	0	
Nuclear grade						
1	1	0	.187	13	1	.311
2	13	0		34	11	
3	31	9		24	4	
Not graded	23	0		1	0	
Radiologic response						
No response	1	1	.401			
Partial response	11	2				
Marked response	7	0				
Complete response	4	0				
No comment	45	6				

Abbreviations: Equiv, equivocal; FS, frozen section; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ITC, isolated tumor cell; LN, lymph node; Macromet, macrometastasis; Micromet, micrometastasis; NAT, neoadjuvant; *P*, *P* value; SLN, sentinel lymph node; +, positive; −, negative.

intraoperative and permanent sections. In non-NAT cases, a larger (T2 or T3) radiological size of the primary BC, SLN metastasis that were ITCs or micrometastasis, and BC with a lobular histologic subtype predicted discordance. In general, results from the NAT patients between the cancer center and community hospital were comparable (results not shown).

The nature of discordances in the NAT patients was separated into sampling-type errors and interpretative-type errors. Sampling-type errors are encountered when the metastatic deposits are not seen on FS but identified on the deeper levels of permanent sections or immunohistochemistry. Interpretative-type errors are metastases present on FS but missed intraoperatively. Of the 9 discordant cases, 7 cases were sampling-type errors only, and 2 cases were both sampling- and interpretative-type errors. Figures 1 and 2 are FS slide images of the 2 discordant cases with interpretative-type errors. In both cases, missed metastasis was associated with tumor bed changes. In terms of outcome, 4 of the 9 false-negative diagnoses went on to have ALND in a subsequent surgery.

Discussion

Lymph node status is one of the most important prognosticators in BC.[11] Axillary lymph node dissection was originally the standard management to assess nodal status; however, it is associated with high morbidity. Sentinel lymph node biopsy

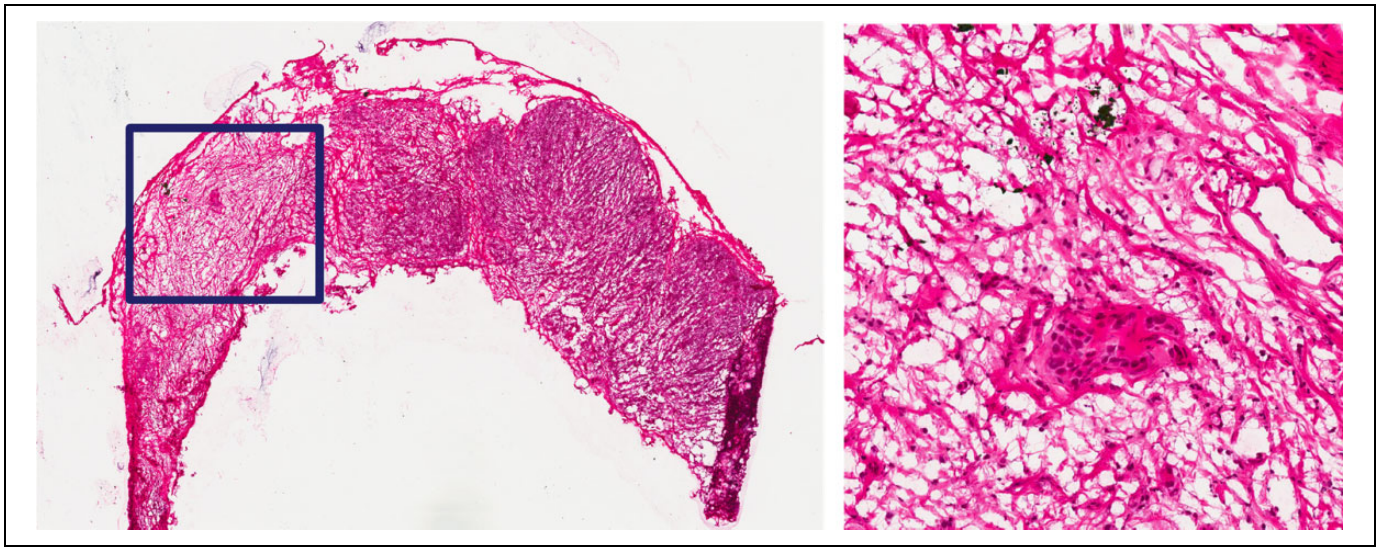

Figure 1. Case 1 demonstrates a focus of isolated tumor cells measuring 0.15 mm that was missed intraoperatively due to its minute size.

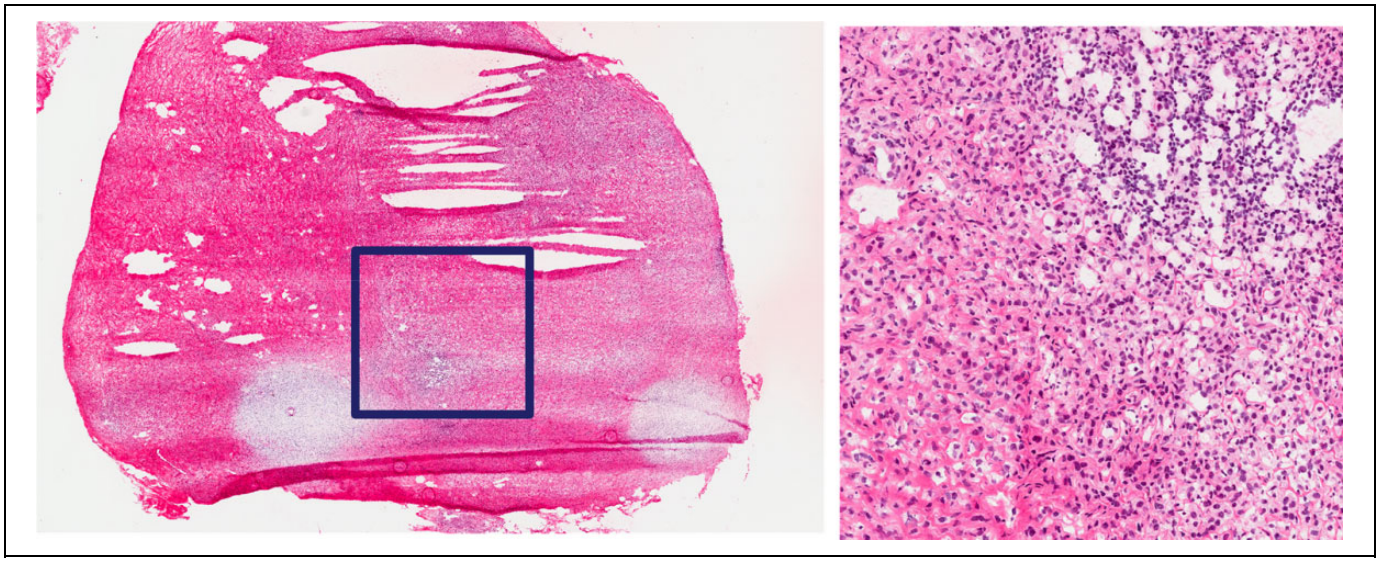

Figure 2. Case 2 demonstrates a focus of macrometastasis measuring 6 mm that was missed intraoperatively due to its lobular growth pattern.

followed by adjuvant radiotherapy in early-stage and limited SLN metastatic disease is the current standard of management in the non-NAT setting.[12] The American Society of Clinical Oncology recently recommended SLN biopsy for NAT patients based on the benefits of avoidance of ALND morbidity and the potential of lymph node downstage following NAT; however, guidelines in the literature on intraoperative assessment of BC in the NAT setting are not well established.[12]

For non-NAT patients, recent studies have demonstrated that FNR for intraoperative assessment of SLN vary from 13% to 22.6%,[13-17] including cases with ITCs. Meanwhile for NAT patients, approximately 50% of patients have residual nodal disease after NAT,[18] and FNR for intraoperative assessment vary from 20% to 26.1%,[7-10] including cases with ITCs. In our multi-institutional study, when ITCs are included in the analyses FNR for non-NAT and NAT are 50% and 28.1%, respectively. When ITCs are omitted from our analyses, FNR improves significantly, with FNR for non-NAT and NAT being 33.3% and 20.7%, respectively. The relationship of ITCs and discordance has been described in the literature on intraoperative SLN assessment.[7-10,13-18] In the non-NAT patients, we found higher number of cases with ITCs present than in the NAT patients, and this affected the overall sensitivity and accuracy. The lower number of cases with ITCs present in NAT patients may represent the effectiveness of systemic therapy in eliminating ITCs. There is importance in identifying ITCs in NAT patients, as it may predict an aggressive population of chemoresistant cells either originating from macroscopic nodal metastasis that has undergone partial response or minimal nodal disease that did not respond to NAT.[19,20] The accuracy

of the intraoperative SLN assessment in NAT patients between community hospital and cancer center was quite comparable. This suggests that FS assessment of NAT SLN can be accurately carried out at both community and cancer centers.

To our knowledge, our study is the first study to analyze in detail clinical, radiological, and pathological features that may predict discordant results in intraoperative FS assessment in both NAT and non-NAT patients. In our study, smaller size of SLN metastasis was associated with false negatives in both NAT and non-NAT patients. Biomarker profile determined on core needle biopsy also demonstrated association with discordance in NAT patients. Of the 9 NAT cases with discordant intraoperative SLN assessment, 8 cases had a biomarker profile of ER-positive and HER2-negative immunophenotype. One case had ER-negative and HER2-negative immunophenotype and had a sampling-type error, where the lesional cells were only identified on deeper sectioning. The propensity for ER-positive and HER2-negative immunophenotype to have discordance can be explained by this biomarker profile's relative resistance to systemic therapy, and thus the tendency to have lymph node metastases refractory to treatment.[21-23] In non-NAT patients, larger radiologic tumor size (T2 or T3) and lobular histotype also significantly predicted false negatives; these findings were not seen in NAT patients. Clinical and radiologic tumor size have been shown to be associated with increased risk of lymph node metastasis, and metastatic carcinoma with lobular histotype is notoriously difficult to identify in an intraoperative setting due to its propensity to grow as single cells and its low nuclear grade.[24,25]

Among the 9 discordant NAT cases, all cases had sampling-type errors and 2 cases had interpretative-type errors as well. FS artifacts such as tissue folding and tissue shattering of adipocytic lymph nodes accounted for some of the sampling-type errors. In addition, some metastases were revealed in deeper sections. Missed metastatic deposits were typically found in areas with tumor bed change consisting of fibrosis, lymphohistiocytic inflammatory infiltrate, and loss of normal lymph node architecture. Good quality FS and careful microscopic examination of the lymph node during intraoperative assessment, especially in areas with tumor bed changes, are essential to limit FNRs due to both sampling- and interpretative-type errors.

It is important to limit false negatives as 4 of the 9 discordant NAT cases went on to have ALND in a subsequent surgical procedure. In terms of outcomes, the need for ALND versus axillary radiotherapy in women with residual metastatic lymph node disease is being examined in the Alliance A011202 trial.[26,27] Recurrence-free period, overall survival, and side effects are also to be studied. While awaiting the results for this trial, the clinical decision for ALND following a false-negative SLN diagnosis varies and often requires a multidisciplinary decision.

Our study has several limitations. Our study is a retrospective nonrandomized study. Therefore, the patient and tumor characteristics of cases treated with and without NAT were different, with the NAT group having younger age at diagnosis, more positive lymph node cytology, larger radiologic tumor size, more ER-negative and HER-positive biomarker profiles, and higher nuclear grade. As ALND post-NAT is still the current standard of treatment for patients with positive LN pre-NAT, with SLN biopsy performed in such patients only starting 2010, our sample size is small.

Conclusion

Detection of SLN metastases by intraoperative FS assessment post-NAT is feasible and demonstrates similar results as in non-NAT setting, although FNR are high in both settings especially with the inclusion of ITCs. Discordance in NAT cases showed statistically significant association with ER-positive/HER2-negative biomarker profile and size of metastatic deposit. The FS SLN biopsy in the community and academic setting shows similar sensitivity, specificity, and accuracy as well as clinicopathological parameters that may predict discordance. Careful examination of lymph nodes and awareness of characteristics that may predict discordance is necessary to avoid high FNR.

Declaration of Conflicting Interests

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