Touch Imprint Cytology and Frozen-section Analysis for Intraoperative Evaluation of Sentinel Nodes in Early Breast Cancer*

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Abstract. *Sentinel lymph node biopsy (SLNB) is currently the suggested axillary staging procedure in patients with early-stage breast cancer (BC) and usually requires intraoperative frozen-section (FS) examination of the removed nodes. However, other techniques, such as touch imprint cytology (IC), real-time reverse transcriptase-polymerase chain reaction and rapid cytokeratin immunostaining on FS may be used. The aim of this preliminary study was to assess the usefulness of intraoperative IC and FS section analysis together in improving the accuracy of sentinel lymph node evaluation in patients with early BC, who underwent SLNB. A series of 126 consecutive women (median age 52, range 34-71 years) with T1 (≤20 mm) BC, were prospectively enrolled in the study. A total of 221 axillary nodes were processed for both IC and FS intraoperative evaluation. Final pathology revealed 74 out of 221 (33.5%) nodes with metastasis, out of which 51 (68.9%) had macrometastases. Overall, 31 out of 126 (24.6%) patients were staged as having pN1mi or pN1a. The sensitivity, specificity, and accuracy in detecting metastases were 75.7%, 100% and 91.9% for FS, 70.3%, 98.6% and 89.1% for IC, and 89.2%, 100% and 96.0% for IC+FS together, respectively. The sensitivity of FS and IC did not differ significantly (p=0.46), while the combination of FS+IC showed a higher sensitivity (p=0.03), and similar accuracy. Our preliminary data confirm that IC is a simple and rapid technique with good sensitivity, suggesting that the combination of FS and IC may be useful in all patients requiring intraoperative SLNB evaluation.*

Sentinel lymph node (SLN) biopsy is currently the suggested axillary staging procedure in patients with early-stage breast cancer (BC) and usually requires intraoperative frozen-section (FS) examination of the removed nodes (1). However, other techniques, such as touch imprint cytology (IC) or scrape cytology, real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and rapid cytokeratin immunostaining (RCI) on FS, may be used (2, 3). The aim of this preliminary study was to assess the usefulness of intraoperative IC and FS section examination together in improving the accuracy of SLN evaluation in patients with early BC who underwent SLN biopsy.

Patients and Methods

A series of 126 consecutive women (median age 52 years, range 34-71 years) with T1 (≤20 mm) BC, detected by fine-needle aspiration cytology (FNAC), core biopsy or open biopsy, and clinically negative axillary nodes (N0), were prospectively enrolled in the study. In patients with nonpalpable lesions, the FNAC or biopsy were performed using wire needle localization, under ultrasound or stereotactic guidance. Written informed consent was obtained from all the participants. All patients underwent SLN biopsy using a combined radioisotope (^{99m}Tc labeled nano-colloid) and the Patent Blue V dye method, as previously reported, according to the American Society of Clinical Oncology guidelines (1, 4). In each patient, one or more SLNs were identified, excised, and sent for pathologal examination. A total of 221 SLNs (median 2, range 1-5 nodes per patient) were processed for intraoperative IC and FS evaluation. The results were compared against the permanent hematoxylin and eosin (H&E) section histology and cytokeratin immunostaining, which were considered as the reference test. A

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Table I. *Comparison of the results of intraoperative standard frozen-section (FS) and imprint cytology (IC) examination with final pathological examination of the overall (n=221) sentinel lymph nodes removed.*

Method	TP	FN	TN	FP	Sensitivity	Specificity	PPV	NPV	Accuracy
IC	52	22	145	2	70.3%	98.6%	96.3%	86.8%	89.1%
FS	56	18	147	0	75.7%	100%	100%	89.1%	91.9%
IC+FS	66	8	147	0	89.2%	100%	100%	94.8%	96.0%

TP, True-positive; FN, false-negative; TN, true-negative; FP, false-positive; PPV, positive predictive value; NPV, negative predictive value; IC+FS, combined IC and FS.

Table II. *Differences between cases of true-positive and false-negative findings on imprint cytology.*

Parameter	True-positive	False-negative	*p*-Value
Number of axillary nodes	52	22	–
Age of the patient (years)	52.4±9.8	51.3±9.6	0.21
Body mass index (kg/m^2)	22.7±3.2	24.0±2.3	0.09
Tumor size (mm)	12.2±3.7	10.5±4.1	0.08
Infiltrating ductal *vs*. other types	52/16	4/2	0.45
G1-2 *vs*. G3	53/15	3/3	0.15
ER≥10% *vs*. ER<10%	48/20	3/3	0.27

G, Tumor nuclear grading; ER, estrogen receptor positivity rate.

positive SLN finding was immediately followed by level I-II axillary clearance, with the aim of sparing the patient a second operation (5).

The excised LNs were bisected if the width was ≤5 mm, or sliced into 1.5-mm thick sections if >5 mm, and touch imprints were made of both surface sections. According to Francz *et al*. (6), one air-dried slide of the imprints was stained with May-Grünwald-Giemsa stain. Two frozen sections (H&E stain, 40 μm between levels), using standard laboratory procedures, were also obtained. Results were immediately communicated to the surgical team. The residual specimens were fixed in 4% neutral-buffered formalin. At a later date, three additional sections (4-6-μm thick) were cut from each face of the formalin-fixed, paraffin-embedded node slab for permanent histology after H&E staining. Anti-cytokeratin was used as the primary antibody, together with a polymer horseradish peroxidase-labeled secondary antibody (DAKO, Glostrup, Denmark) (3, 7).

Sensitivity was defined as true-positives (TP)/TP+false-negatives (FN), specificity as true-negatives (TN)/TN+false-positives (FP), positive predictive value (PPV) as TP/(TP+FP), negative predictive value (NPV) as TN/(TN+FN), and accuracy as (TN+TP)/overall number of patients. The chi-squared (χ^2) test and the Student's *t*-test were used to compare the results. The reported data are expressed as the mean±standard deviation (SD) and *p*-value <0.05 was considered statistically significant.

Results

Invasive carcinoma was diagnosed as ductal in 98 (77.8%) patients, combinations of infiltrating ductal and other types in 12 (9.5%) patients, lobular in 11 (8.7%), adenocystic in three (2.4%), and papillary in two (1.6%) patients. Final pathology revealed 74 out of 221 (33.5%) axillary LN to have metastases, out of which 51 (68.9%) had macrometastases. Overall, 31 out of 126 (24.6%) patients were staged as pN1mi or pN1a, according to the AJCC staging (8).

The sensitivity, specificity, PPV, NPV and accuracy of IC, FS, and combined IC and FS in detecting axillary LN metastases are reported in Table I. The sensitivity of FS and IC did not differ significantly (χ^2=0.55, p=0.46), while the combination of FS and IC together had a higher sensitivity (χ^2=4.67, p=0.03), at the same specificity and similar accuracy (91.2% *vs*. 96.0%: χ^2=3.20, p=0.07). All but one of the FN results corresponded to axillary LNs with micrometastases. Table II shows the differences between cases of TP and FN findings on IC. No significant differences were found between subgroups.

Discussion

In patients undergoing SLN biopsy, FS and IC are the most commonly used techniques. Unfortunately, the reported sensitivity varies widely, ranging from 44% to 100%, and from 33% to 96%, respectively, at a specificity range of 98-100% (3, 9-11). Imprint cytology alone accurately predicts final LN status in up to 85% of patients, according to tumor type and stage (12). Molecular techniques, such as one-step nucleic acid amplification, PCR and RCI, have an overall sensitivity ranging from 78% to 100%, having the potential to eliminate sampling errors (13, 14). However, although some genes (*i.e*. mammoglobin 1, cytokeratin 19) are

expressed in the majority of BC cases, two or more genes should be tested together (10).

In 1999, the College of American Pathologists recommended the use of intraoperative cytological examination to evaluate the SLN (15). When compared with permanent histology, the specificity of FS is close to 100%, but this technique remains expensive, labour-intensive and operator-dependent (10). Furthermore, the process of preparing FS leads to tissue loss, which could result in an understaging of the disease. Using cytological techniques, such as IC and scrape cytology, the cut surface of the LN is pressed or scrape on to a glass slide, stained and examined. However, the difference in total charges between the FN and TP groups outweighs the cost of IC, potentially reducing hospital stay and sparing the patient a second operation, and thus IC represents a cost-effective evaluation of patients with BC (16). It has also been reported that the FN results are more common in the presence of micrometastatic disease and invasive lobular carcinoma, but are reduced by using RCI (13-17). In a prospective study comparing IC, FS and RCI, only the combination of FS and RCI was statistically superior to IC alone, having results comparable to those of permanent section examination (18). Another study showed that the sensitivity and specificity of IC were similar to that of FS evaluation in patients with lobular carcinoma, and no statistically significant differences in accuracy were found in detecting metastases from lobular and ductal carcinoma, while in the presence of micrometastases the sensitivity of both techniques was lower (19). We found an overall sensitivity of 70.3%, 75.7% and 89.2% ($p<0.05$) with IC, FS, and IC plus FS in combination, respectively. In our experience few patients (8.7%) had pure lobular carcinoma. The clinical prognostic significance of micrometastases in SLN remains controversial, and some authors consider micrometastases to behave somewhat similarly to macrometastases, and treat such patients as node-positive (20, 21). Moreover, intraoperative IC is quick to perform, preserves LN tissue for subsequent histological examination and should be considered a cost-effective adjunction to SLN biopsy (12, 22).

Conclusion

In accordance with previous studies (12, 13), our preliminary data confirmed that IC is a simple and rapid technique with a good sensitivity for the detection of macrometastases, and suggest that the combination of FS and IC may be useful in all patients with early BC and clinically-negative axillary LNs who requite intraoperative evaluation of SLN. Moreover, SLN biopsy should also be considered as the standard procedure for axillary LN staging in patients with multicentric BC (23). The remaining axillary micrometastases do not increase the axillary recurrence rate and it is doubtful that those cancer cells are capable of completing the multistep metastatic process (24).

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