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Intraoperative Imprint Cytology of Sentinel Lymph Node in Breast Cancer After Negative Preoperative Ultrasound Assessment of Axilla *How Many Second Operations Are Avoided?*

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OBJECTIVE: Intraoperative imprint cytology (IOIC) of sentinel lymph node (SLN) allows axillary surgery in one sitting in patients with positive SLN, but a second operation may then become necessary to clear margins of the primary tumor. Axillary ultrasound now identifies approximately half the node-positive axillae, reducing the need for intraoperative testing. We studied how many second operations were avoided by IOIC of the SLN.

STUDY DESIGN: Large district general hospital breast unit, retrospective review.

RESULTS: We reviewed 491 patients with negative preoperative axillary ultrasound who underwent SLN biopsy and IOIC over an 8-year period. A total of 108 patients (22%) had macrometastasis in the SLN, and 81 (75%) were diagnosed intraoperatively and underwent axillary clearance in the same sitting. However, 13 of those 81 patients required further surgery to get adequate margins for primary tumor.

CONCLUSION: Only 22% of patients in this study had positive sentinel lymph nodes in the axilla. The false neg-

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ative rate of IOIC was 25%. 16% of patients whose node positivity was intraoperatively diagnosed still required further surgery for primary tumor. IOIC helped to avoid 81 cases of delayed axillary clearances and 68 reoperations in 491 patients. Imprint cytology is inexpensive and quick and remains a worthwhile addition to breast cancer surgery. (*Anal Quant Cytopathol Histopathol* 2018;40:57–62)

Keywords: axillary block dissection, breast cancer, breast neoplasms, cytodagnosis, intraoperative imprint cytology, sentinel lymph node biopsy.

In invasive breast cancer, a sentinel lymph node (SLN) that is positive for macrometastatic disease usually leads to an axillary clearance requiring a second visit to the operating theater. Clinical assessment of the axilla lacks sensitivity and specificity for nodal involvement, but ultrasound (US) with fine needle aspiration cytology (FNAC) or core biopsy of abnormal lymph node under US guidance identifies approximately 50% of the node-positive axillae preoperatively.^{1–3} This leaves a relatively small group of patients in whom node positivity would be diagnosed only by SLN biopsy. These patients usually return to the operating theater for a delayed axillary clearance.

Intraoperative testing of SLN allows patients with positive axillae to undergo axillary clearance in the same sitting, avoiding a second axillary operation. Touch imprint cytology, frozen section, and RNA-based methods can be used intraoperatively and differ in terms of cost, time required, and sensitivity.⁴

Approximately 70% of early breast cancer patients undergo breast-conserving surgery, and 20–30% of those patients will return to the operating theater for further surgery for inadequate margins.^{5,6} In these patients intraoperative analysis of the SLN is not effective in preventing a second operation.

The aim of this study is to see how many returns to the operating theater for further surgery are avoided by intraoperative imprint cytology of the SLN.

Materials and Methods

We reviewed the data on patients with early invasive breast cancer confirmed by core biopsy and who had a negative preoperative assessment of axilla using US +/- US-guided FNAC followed by a successful SLN biopsy and intraoperative assess-

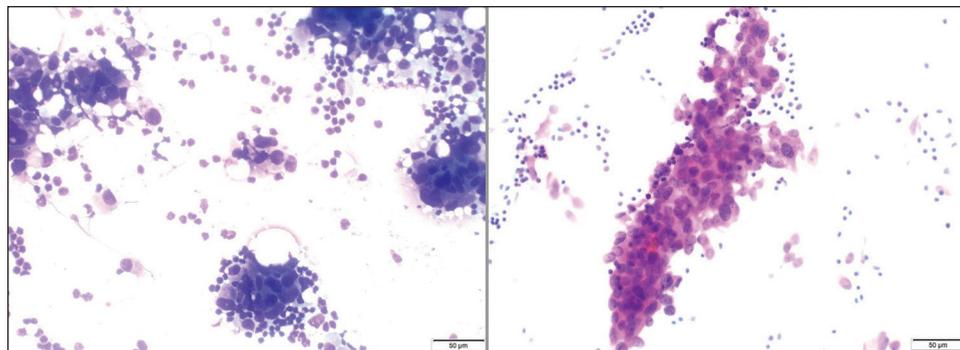
ment of the SLN by imprint cytology, in one breast unit over a period of 8 years.

Axillary US was done at the time of the diagnosis of primary breast cancer. Axillary lymph nodes were assessed for the shape and the morphology of the cortex and classified as abnormal if their longitudinal to transverse ratio is <2 and/or the cortex was concentrically or eccentrically thickened to >2 mm.⁷ When an abnormal-looking node is seen, an US-guided FNAC was performed. In case of multiple abnormal nodes the most abnormal node in the radiologist's opinion was sampled.

Patients with a positive FNAC for metastatic disease underwent axillary clearance. Others underwent SLNB using a combination of patent-blue V dye and radioisotope. Intraoperative imprint cytology was performed when an experienced cytopathologist was available. The SLN was then bisected along the longest diameter, and both cut surfaces were touched onto glass slides. A minimum of 3 smears were taken for each patient. In case of multiple SLNs the bluest/hottest node(s), in the surgeon's opinion, were sampled. The smears were fixed rapidly in alcohol, subjected to quick staining process using Diff-Quik (Midlantic Biomedical, Inc., New Jersey, USA) rapid method, and examined under the microscope without cover slip. The results were categorized as either positive or negative. At least 6 groups or clusters of malignant epithelial cells were considered as minimum requirement for a positive diagnosis (Figure 1). Less than this number were given an indeterminate score. The results were telephoned to the surgeon intraoperatively. Other slides were subsequently stained with standard hematoxylin and eosin stain and compared with the Diff-Quik stained slides (Figure 1).

If the imprint was positive, an axillary clearance was performed. In cases of indeterminate or negative results no further axillary surgery was done. The SLN underwent routine histopathological processing; the bisected node was paraffin sectioned at 2-mm intervals and stained with hematoxylin and eosin. Immunohistochemical stains were used in addition when considered necessary by the pathologist. Results were reported a week after the surgery.

Those with macrometastases in the SLN missed by imprint cytology were recalled for axillary clearance. Those with isolated tumor cells were not offered further surgery, and those with micrometastases were discussed in the Multidisciplinary Team (MDT) and occasionally offered axillary clearance.

**Figure 1**

Left: positive smear from an invasive ductal carcinoma (Diff-Quik stain, $\times 20$).
Right: standard H&E stain to illustrate the same invasive ductal carcinoma (H&E, $\times 20$).

Primary breast cancer surgery (breast-conserving surgery or mastectomy with or without immediate reconstruction of the breast) was performed at the same time as SLN biopsy. Intraoperative specimen radiography was performed on all breast-conserving surgery specimens, and cavity shavings were performed if margins were considered to be close on palpation or radiography. During the study period patients with radial margins < 2 mm on final histology after breast-conserving surgery were recalled for re-excision after MDT discussion if there was more tissue available for re-excision.

Results

Over an 8-year period from January 2007 to December 2014, 491 patients with early invasive breast cancer underwent SLN biopsy and intraoperative imprint cytology following a negative preoperative US assessment of axilla. These patients constitute the study population. Details of diagnosis and other relevant patient and tumor information are presented in Table I.

A total of 122 patients (25%) had metastatic disease in the axilla: 108 macrometastases (22%) and 14 micrometastases (3%). Median number of positive nodes was 1 (range 1–22). The outcome of imprint cytology in relation to SLN metastatic status is presented in Table II. Imprint cytology diagnosed 75% (81/108) of SLN with macrometastatic disease and 14% (2/14) of SLN with micrometastatic disease. Out of 9 indeterminate imprints in the study, 3 had macrometastatic and 2 had micrometastatic disease; the other 4 patients had a negative SLN. There were no false positive imprints, but the 2 patients with micrometastatic disease in the axilla with positive imprints had axillary clearance in the same sitting.

The reasons why patients returned to the oper-

ating theater for further surgery are presented in Table III in relation to imprint cytology results. All positive imprint patients ($n=83$) had axillary clearance in the same sitting, but 13 (16%) of those patients needed a second operation for primary tumor. Among 27 patients with macrometastatic disease in the SLN missed by imprint, 24 returned for axillary clearance; 1 was treated with axillary radiotherapy, and 2 had no further axillary surgery following MDT discussion.

Among 12 patients in whom SLN micrometastases was missed by imprint cytology, 3 had axillary clear-

Table I Patient, Ultrasound, Tumor, and Operative Details ($n=491$)

Mode of diagnosis of breast cancer	
Symptomatic	274
Screening	217
Median age (range)	61 (25–90)
Median preoperative tumor size in mm (range)*	15 (5–50)
Preoperative axillary US / FNAC results	
Normal nodes, FNAC not done	401
Abnormal nodes, FNAC done	90
Results of US-guided FNAC of lymph node ($n=90$)	
Inadequate for diagnosis (C1)	12
Benign (C2)	78
Surgery for primary breast cancer at the time of SLN biopsy	
Breast conserving surgery	396
Mastectomy	82
Mastectomy with reconstruction	13
Median no. of SLNs (range)	2 (1–10)
Final tumor histology	
Invasive ductal carcinoma	372
Invasive lobular carcinoma	69
Mixed ductal and lobular	18
Other histological subtypes	32
Median final tumor size in mm (range)	17 (2–100)

*27 patients had multifocal cancers.

Table II Outcome of Intraoperative Imprint Cytology in Relation to Sentinel Lymph Node Histology

	Imprint positive	Imprint negative	Imprint indeterminate
SLN positive for macrometastases (n=108)	81	24	3
SLN positive for micrometastases (n=14)	2	10	2

ances (1 with mastectomy) and 1 returned for re-excision of margins. Others had no further surgery.

False negative rate of imprint cytology for macrometastatic disease was 25%. Overall, 18% of patients (70/396) who had breast-conserving surgery required further surgery for primary tumor. Assuming that 2 patients with SLN micrometastases and positive imprints who had clearances may not have had an axillary clearance if intraoperative testing was not done, intraoperative imprint cytology done in 491 patients helped to avoid 68 reoperations in total.

Discussion

The number of second operations prevented by intraoperative testing of SLN would depend on the prevalence of node positivity in the population studied, the re-excision rates following breast-conserving surgery, and sensitivity of the intraoperative test used. As axillary US now identifies half the node-positive axillae preoperatively, the node positivity rate in patients undergoing SLNB has dropped significantly; only 1 in 4 women with an US-negative axilla would have a positive SLN, and in our study this was only 22% for macrometastases.²

Table III Reasons for Reoperation in Relation to Results of Intraoperative Imprint Cytology

Result of intraoperative imprint cytology	Return to operating theater		
	Excision of margins/mastectomy	Axillary clearance	Both
Negative (n=399)	46	12	9
Positive (n=83)	13	0	0
Indeterminate (n=9)	1	2	1
Totals	60	14	10

There is no consensus as to what is an adequate margin in breast cancer, which is partly responsible for the large variation in the re-excision rates reported.⁸ Our re-excision rate of 18% compares well with a rate of 20–30% reported in the literature.^{5,6} Gross palpation of the specimen, intraoperative specimen radiography, and taking “cavity shavings” (all used in this study), as well as intraoperative margin assessment using imprint cytology, frozen section, and other methods appear to reduce (but not abolish) the re-excision rates.^{9,10} Some of these methods will result in significant lengthening of the operation time. A recent meta-analysis suggests that while tumor reaching the excision margin is associated with an increased risk of local recurrence, wider margins are not associated with significantly less recurrence rates.¹¹ There is an emerging view that the risk of local recurrence after breast cancer surgery is related to tumor biology rather than the width of the excision margin.

In this study, imprint cytology had a sensitivity of 75% with a specificity of 100%. Avoidance of false positives is critical, and indiscriminate use of indeterminate category defeats the purpose of the procedure. A review of published studies shows that while the specificity of imprint cytology approaches 100%, its sensitivity (69–81% for macrometastases) is lower than that of frozen section (up to 92%) or RNA-based methods (up to 93%).^{4,12,13} Micrometastatic disease, which is of doubtful clinical significance, and invasive lobular carcinoma (ILC) where the metastatic cells resemble normal lymphoid cells (Figure 2) are more prone to be missed. The problem with ILC was overcome in this study by comparing the core needle biopsy slides of the primary tumor with the imprint cytology sample. Taking slides from multiple cut sections of the SLN and rapid immunocytochemistry can improve the sensitivity of imprint cytology but will also increase the time needed for reporting.

There have not been many published studies looking at the role of intraoperative testing of SLN after negative US assessment of axilla, but 2 studies using frozen section and imprint cytology after negative preoperative US have suggested that reoperation is avoided in only 4–9%, but the imprint cytology study had very poor sensitivity (45%).¹⁴⁻¹⁶ However, with a much higher sensitivity (75%) we found imprint cytology a much more suitable test for intraoperative testing in a busy district general hospital. It takes less than 10 minutes, so the results are usually reported by the time the surgeon had

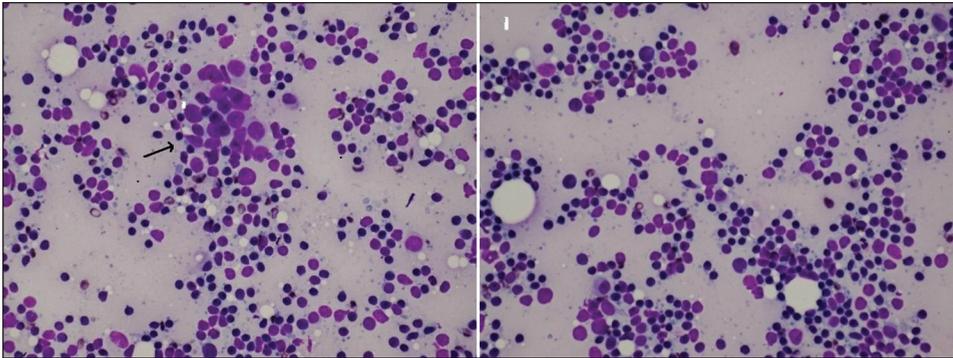


Figure 2

Left: smear showing a cluster of malignant cells from an invasive lobular carcinoma (arrow) (Diff-Quik, $\times 10$). Right: a negative smear demonstrating the difficulty in discriminating the normal lymphocytes from lobular carcinoma cells shown on the left (Diff-Quik, $\times 10$).

completed surgery for the primary tumor (breast-conserving surgery or mastectomy). It costs very little and there were no false positives, but it does require a pathologist with experience in both the staining technique and reporting breast cytology.

One recent clinical trial suggests that a proportion of SLN-positive patients (those with T1-T2 tumors and 1–2 positive SLNs) having breast-conserving surgery (and subsequent whole-breast radiotherapy and adjuvant systemic therapy) may not need axillary clearance.¹⁷ It has also been suggested that a margin “where the tumor cells do not touch the ink” is acceptable.¹⁸ If these approaches are deemed safe by further studies and clinical practice and are widely adopted, fewer breast cancer patients would require a second operation. Intraoperative testing of SLN would also become less relevant in these patients as finding cancer in 1–2 SLNs will not lead to an axillary clearance.¹⁹

In conclusion, intraoperative imprint cytology performed in 491 patients with US-negative axilla helped to avoid 81 cases of delayed axillary clearances and 68 reoperations. Imprint cytology is inexpensive and quick, and it remains a useful undertaking in breast cancer surgery at present.

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