Imprint Cytology of Sentinel Lymph Nodes in Breast Cancer

Experience with Rapid, Intraoperative Diagnosis and Primary Screening by Cytotechnologists

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OBJECTIVE: To evaluate the intraoperative imprint diagnoses of smears from sentinel lymph nodes that had been primary screened by cytotechnologists and to assess the most important causes of false negative (FN) imprint diagnoses.

STUDY DESIGN: Material consisted of 429 imprints from sentinel lymph nodes in 211 breast cancer patients that were sent for frozen section examination over 13 months.

RESULTS: The mean number of imprints/lymph nodes per patient was 2.02. The mean screening time per imprint was 3.6 minutes. Sixty-six sentinel nodes (16%) from 51 women (24%) were metastatic. Imprints and/or frozen sections were positive in 54 nodes (82%). Imprints were positive in 38 nodes, representing 70% of intraoperative positive nodes and 58% of the total number of positive nodes. Twenty-six of 28 (93%) FN imprints were due to suboptimal sampling. Four of 9 FN macrometastases did not contain diagnostic or suspicious cells/cell groups even on rescreening, whereas a few, and then only 1 diagnostic group were identified in 2/9. There were no false positives.

CONCLUSION: Primary screening by experienced cytotechnologists is both rapid and reliable and enabled the diagnosing pathologist to concentrate on the frozen section. The major cause of false negative imprints is sampling, even in macrometastases. (Acta Cytol 2003;47:768–773)

Intraoperative imprint cytology of sentinel nodes in breast cancer is rapid and, when positive, reliable.

Keywords: sentinel lymph node biopsy, breast cancer, imprint cytology.

Axillary lymph node status is essential in staging breast cancer but unfortunately has relatively high morbidity. The technique of sentinel node (SLN) biopsy was developed to assess axillary nodal sta-

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tus without removing most of the axillary contents.[1-9] SLN is defined as the first node in the lymphatic basin that receives the primary lymphatic flow. When the SLN is tumor free, the risk that any other axillary node is involved is virtually nonexistent,[10] and axillary dissection can be omitted. The SLN can be examined intraoperatively as both a frozen section and touch imprint smears. Some studies have found considerably lower sensitivity of imprint diagnoses than frozen sections,[11-14] whereas others have found it comparable to that of frozen sections.[15-17]

Intraoperative touch preparations from a variety of anatomic sites are used in a number of institutions in conjunction with frozen sections.[18-21] As compared to frozen sections, imprints usually reveal better cellular detail and fewer artifacts. Accuracy rates are high,[18,20,22] and may rival those of frozen section.[18-20]

The technique of SLN biopsy was introduced as a routine procedure in our institution in April 2000 after evaluation of a test period during which the SLN biopsy was located in 75 breast cancer patients who subsequently underwent axillary dissection. Both frozen sections and touch imprints were examined. Serial sectioning and immunohistochemical analysis of AE-1/AE-3 were done in all primary node-negative cases. In that pilot study, we found equal sensitivity of imprints and frozen sections (unpublished results). To reduce the time and relieve the cytopathologist, we decided to train 4 experienced cytotechnologists (CTs) to do the primary screening of imprints. The aim of this study was to evaluate the intraoperative imprint diagnoses of smears that had been screened by CTs and to assess the most important causes of false negative imprint diagnoses.

Materials and Methods

The material consisted of 429 imprints from sentinel lymph nodes of 211 breast cancer patients that were sent for frozen section examination over 13 months. All nodes were cut in 2 and eventually 3 parts, and imprints were made from all cut surfaces. The smears were air dried and stained with Diff-Quik (Dade, Dudingen, Switzerland).

Primary screening was done by 4 CTs with 16–31 years (mean, 24) of experience screening all kinds of cytologic specimens. The CTs reported their results directly to a cytopathologist, who examined both the imprint and corresponding frozen section. A written CT report containing the CTs' evaluation of the smears as well as the screening time was filed. Suspicious findings were filed as negative.

Two or 3 frozen sections were cut eventually from each cut surface, usually 4–6 sections per node. The same was done with the embedded rests of the frozen sections, and normally 8–12 sections were examined per lymph node. Serial sectioning, including immunohistochemistry, was not done routinely.

Primary screening by specially trained and experienced cytotechnologists is a valuable and time-saving option.

The combined results of imprint and frozen section were reported back to the operating surgeon. Suspicious but not diagnostic findings were reported as negative.

For a short period of time, rapid immunocytochemistry (ICC) (Envision, Dako, Glostrup, Denmark) of imprint and frozen sections was tested. The majority of breast cancers operated on at our hospital are screening detected, and the percentage of positive axillary lymph nodes is 21.5%.[23]

All false negative imprints and frozen sections were rescreened by the first author, and the diameter of missed metastatic foci was recorded. Diagnostic or suspicious cells or cell groups found at rescreening were reviewed once more. The cases were discussed at a consensus meeting using a multiheaded microscope and diagnostic cases recorded as interpretation (numerous diagnostic cells/groups but not recognized as such) or detection error (few diagnostic cells or cell groups but diagnostic when seen).

Results

The mean number of imprints/lymph nodes per patient was 2.02. The mean screening time per imprint was 3.6 minutes. Totally, 66 sentinel nodes (16%) from 51 women (24%) were metastatic. The details of the results are given in Table I. Imprints and/or frozen sections were positive in 54 nodes (82%). The rest, 12 nodes from 11 women, were diagnosed on the permanent, embedded sections. The true false negatives represented 7 women (3.3%)

Table I *Overview of Results of Imprints, Frozen Sections and Permanent, Embedded Sections*

Result	No. of lymph nodes	No. of patients
Imprint, frozen section and permanent, embedded section positive (Figures 1 and 2)	32	24
Imprint negative, frozen section and permanent, embedded section positive	16	14
Imprint positive; frozen section positive; permanent, embedded section negative	1	1
Imprint positive; frozen section negative; permanent, embedded section positive (Figures 3 and 4)	2	2
Imprint positive; frozen section and permanent, embedded section negative	3	2
Imprint and frozen section negative; permanent, embedded section positive	12 (18%)	11
Imprint, frozen section and permanent embedded section negative	363	160

with only 1 or, if more, only negative additional sentinel nodes. These patients had to undergo a second operation. Six were axillary dissections only, whereas the last was a breast resection (margins not free in the primary surgical specimen) and axillary dissection.

Imprints were positive in 38 nodes, representing 70% of intraoperative positive nodes and 58% of the total number of positive nodes. The frozen sections were positive in 51 nodes, representing 94% of intraoperative positive nodes and 77% of the total. There were 28 false negative imprints (42%). As suspicious cases were registered as negative, the sensitivity of the 2 modalities was 58% and 77%, respectively. There were no false positives, and the specificity was 100%.

The results of the rescreening of the false negative imprints and frozen sections are given in Tables II and III. The mean and median diameters of metastatic foci missed by both imprint and frozen section were 1.8 and 1.5 mm, respectively. The mean and median diameters of metastases missed by imprint only were 4.6 and 3 mm.

In 3 lymph nodes from 2 women, only the imprints were positive, whereas both frozen sections and the permanent, embedded sections were negative. However, both women had several other positive lymph nodes that were confirmed by both frozen section and the permanent, embedded sections. In 2 women whose sentinel nodes were imprint positive/frozen section negative, the axillary dissection was done on the cytologic diagnosis alone (4%). (One of them is illustrated in Figures 3 and 4.) The permanent, embedded sections confirmed the metastatic lesions.

Rapid ICC did not add any information in the examination of sentinel nodes during the testing period. The vast majority of cases were negative, and only obvious positive cases were ICC positive.

Discussion

The sensitivity of intraoperative sentinel imprints

Table II *Details of Cases with False Negative Imprints and Positive Frozen Sections*

Case	Imprint	Metastatic focus (mm)	Error type	Node status
Ductal pT1c G1[a]	Few single cells	1.5	Sampling/detection	2/7
Lobular pT2 G2	1 Diagnostic group	1.5	Sampling/detection	1/9
Ductal pT1c G1	Negative	4	Sampling	1/17
Tubular pT1a G1	Negative	1	Sampling	2/10
Ductal pT1b G1	Negative	<0.5	Sampling	1/10
Ductal pT2 G1	Few diagnostic groups	>10	Sampling/detection	9/16
Ductal pT2 G1	Numerous diagnostic groups	>10	Interpretation	9/16
Ductal pT2 G1	Several diagnostic groups	7	Interpretation	9/16
Ductal pT1c G2	Negative	3	Sampling	2/14
Ductal pT2 G1	Negative	2	Sampling	2/11
Ductal pT2 G1	Negative	2	Sampling	1/11
Ductal pT1c G2	1 Diagnostic group	6	Sampling/detection	1/11
Ductal pT2 G3	1 Equivocal cell group	4	Sampling	2/12
Ductal pT1c G2	Negative	>20	Sampling	1/3
Ductal pT1b G1	1 Equivocal cell group	1.5	Sampling	2/22
Ductal pT2 G2	1 Diagnostic group	1.5	Sampling/detection	1/16

[a]Same case in Tables II and III. There were 2 SLNs with different findings, as shown in the 2 tables.

Table III *Findings in Rescreened False Negative Imprints and Frozen Sections*

Case	Imprint	Frozen section (mm)	Metastatic focus (mm)	Error type	Node status
Ductal pT2 G3	Negative	Negative	< 0.5	Sampling	2/12
Ductal pT1c G2	2 Small groups	< 0.5	2	Sampling/detection	1/10
Ductal pT1c G1	Too scant cell material	< 1	1	Sampling	1/2
Ductal pT1c G1	Negative	Negative	< 0.5	Sampling	1/17
Ductal pT1c G3	Negative	Negative	1.5	Sampling	1/16
Ductal pT1b G3	1 Equivocal group	Negative	< 1	Sampling	2/12
Ductal pT1b G3	Negative	Negative	< 1	Sampling	2/12
Ductal pT1c G1	Negative	Negative	5	Sampling	2/12
Ductal pT2 G2	Negative	Negative	1.5	Sampling	1/16
Ductal pT2 G2	Few but diagnostic tumor cell groups	Negative, poor quality	2	Sampling/detection	1/10
Ductal pT1c G1[a]	Scant cell material	1	2 Foci, 1 and 1.5	Sampling	2/7
Ductal pT1c G2	Few but diagnostic tumor cell groups	4	4	Sampling/detection	1/2

[a]Same case in Tables II and III. There were 2 SLNs with different findings, as shown in the 2 tables.

in our study was considerably lower than the sensitivity of frozen sections. Our results are in the same range as are some other studies.[11-14] Sampling error with no detectable carcinoma cells or cell groups on rescreening was the cause of the false negative results in 18 lymph nodes. In another 8 nodes the main cause was also sampling, but in addition a few diagnostic tumor cells on the smears had been missed at primary screening. Totally, 26/28 (93%) false negative imprints were due to suboptimal sampling. It is not surprising that both frozen sections and imprints missed some of the micrometastases (metastatic foci of ≤2 mm[24]). What is surprising and also quite disappointing is that sampling from metastases as large as 10–20 mm did not yield any or only rare tumor cell groups or single tumor cells. From Table II we can see that 4/9 macrometastases did not contain diagnostic or suspicious cells/cell groups even on rescreening, whereas a few and eventually only 1 diagnostic group were identified in 2/9. What could be the reason? Only 2 of the false negative imprints were scant in cellularity. From our fine needle aspiration cytology practice we are used to thinking that carcinoma cells have variable but generally reduced cell cohesion. However, in comparison to the lymphoid cells in the nodes, the cell cohesion is distinct, especially in many low grade carcinomas. In addition, some of the metastatic foci, especially macrometastases, had a fibrous stroma, which apparently makes detachment of carcinoma cells more difficult.

Blumenfeld et al[25] compared the cellularity of material obtained by aspiration, touch and scraping. Scrape preparations yielded the most cellular specimens. Theoretically, scraping off material from the cut surface of the lymph nodes should yield more diagnostic tumor cells.[13,16] In 2 studies the sensitivity was 82% and 67.7%.[13,16] Thus, even if scraping might improve the diagnostic cell yield, it does not solve the problem.

Some micrometastases will be missed either on imprint, frozen section or both due to their small size and the location of the metastatic foci in the nodes. Most papers[11-13,16] report that >75% (range, 75–99%) of false negative imprints were from micrometastases. Our rate of 68% micrometastases falls just below that (Tables II and III).

Interpretation error occurred in only 2 lymph nodes from 1 woman when a number of diagnostic carcinoma cell groups were found on rescreening. In the 8 lymph nodes in which a few diagnostic or suspicious groups were found on rescreening, they most probably had not been noticed during primary screening. From cervical screening it is well known that if the number of abnormal cells is small, there is a distinct risk that the cells will not be detected. Possibly we also have the same psychological effect as in cervical screening, that the primary screener does not expect to find anything in the large majority of cases.

A large number of sentinel nodes had distinct or extensive sinus histiocytosis, and most of the cell groups interpreted as suspicious by the primary screeners were aggregates of histiocytic cells. None of the "suspicious" specimens were metastatic on the frozen or permanent sections.

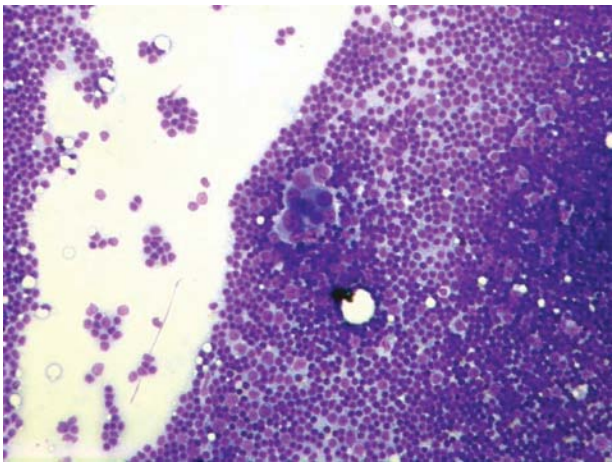

Figure 1 Diff-Quik–stained imprint. Lymphoid cells with a single group of carcinoma cells (×250).

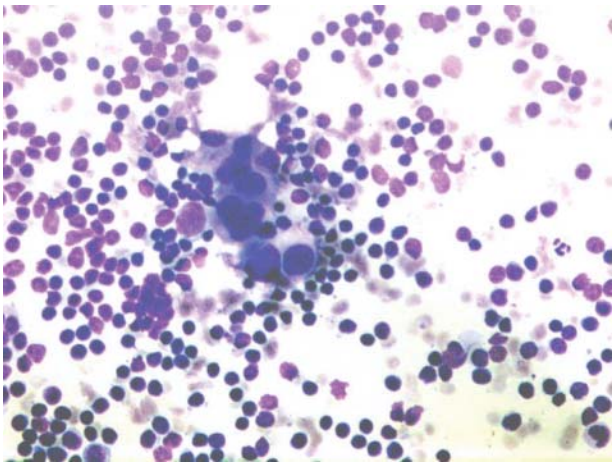

Figure 3 Diff-Quik–stained imprint with loosely cohesive group of carcinoma cells (×400).

Primary screening by experienced cytotechnologists enabled the diagnosing pathologist to concentrate on the frozen section. In obviously positive cases, the imprint diagnoses were ready before the frozen sections, and the time for reporting the diagnosis to the operating surgeon could be shortened. Positive imprints with corresponding negative frozen sections occurred in a few cases but were decisive for further surgical action in only 2 women.

Some[11-13] advocate the use of intraoperative imprints only and no frozen section examination to avoid disturbing artifacts in the permanent sections. At our institution that would lead to another operation under general anesthesia in 30% of the SLN-positive women, and that is not acceptable.

In conclusion, intraoperative imprint cytology of sentinel nodes in breast cancer is rapid and, when positive, reliable. Primary screening by specially trained and experienced cytotechnologists is a valuable and time-saving option. The main problem is sampling, even in macrometastases. Just as in cervical cytology, smears containing few diagnostic cells or cell groups might be missed at primary screening. The sensitivity is lower than for frozen sections, and, in our opinion, imprint cytology should not be used alone but in conjunction with frozen sections.

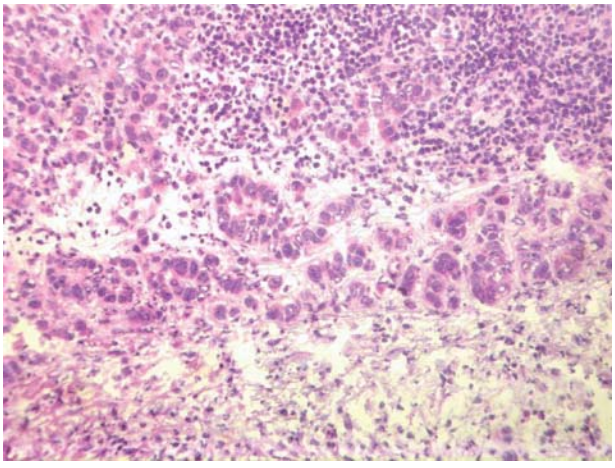

Figure 2 Corresponding hematoxylin-eosin–stained frozen section with metastatic area (×150).

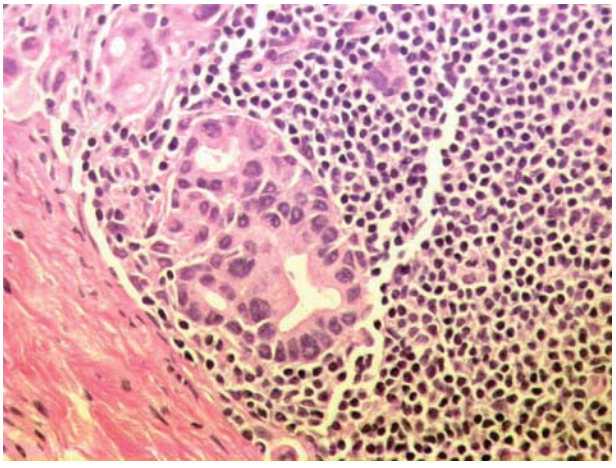

Figure 4 Corresponding hematoxylin-eosin–stained permanent section (×250).

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