Intraoperative Examination of Sentinel Lymph Nodes by Ultrarapid Immunohistochemistry in Breast Cancer

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Background: The ultrarapid immunohistochemistry (IHC) technique was applied to the intraoperative examination of sentinel lymph nodes (SLNs) because routine SLN frozen section examinations sometimes produce false-negative results. The present study was undertaken to develop a reliable protocol for the ultrarapid IHC of SLNs.

Methods: SLNs from 79 breast cancer patients with clinically negative axillary node were examined intraoperatively by frozen hematoxylin–eosin (H&E) stain and by ultrarapid cytokeratin IHC assay. On the basis of the result of serially sectioned permanent study, the sensitivity and accuracy of each intraoperative technique were compared.

Results: The total number of dissected SLNs was 178 with a mean of 2.3 (1–5) per patient. The mean turnaround time for ultrarapid IHC was 20 min. The sensitivity rates of frozen H&E staining and ultrarapid IHC were 70.0 and 85.0%, respectively \((P = 0.083)\). Each method had a specificity of 100%. The accuracy rates for frozen H&E staining and rapid IHC were 92.4 and 96.2%, respectively \((P = 0.083)\). Ultrarapid IHC detected one additional patient with sentinel node micrometastasis and two additional patients with isolated tumor cells (ITCs). In those patients, two underwent completion axillary dissection simultaneously and could avoid a second operation.

Conclusions: Ultrarapid cytokeratin IHC enhanced the intraoperative detection of sentinel node micrometastasis and ITCs in breast cancer without consuming much time. In patients who need completion axillary dissection after sentinel node biopsy, this technique could be helpful in avoiding a second operation.

Key words: breast cancer – micrometastasis – sentinel lymph node – ultrarapid immunohistochemistry (IHC)

INTRODUCTION

The status of axillary lymph nodes, tumor size, histologic grade, hormonal receptor status and DNA index are prognostic factors in breast carcinoma, and the status of axillary lymph nodes remains the single most important prognostic factor and the most important factor when deciding on adjuvant chemoradiation therapy and for predicting outcome (1,2). Axillary dissection is still the gold standard for staging axilla and for local control and provides lowest false-negative (FN) frequencies and local recurrence rates (3). However, severe axillary neurovascular injury sometimes occur and lymphoedema, pain, and arm motion difficulties are common; moreover, these are associated with the extent of dissection. The early detection of breast cancer increased as mammographic screening became more widespread, and the proportion of patients with negative axillary nodes has increased (4). In cases of clinically node-negative patients, axillary nodes were found to be histologically positive only in \(~12\%\) and \(25–47\%\) revealed only micrometastasis. These patients did not derive any therapeutic benefit from axillary dissection, but could experience significant morbidity as a result of the procedure. The desire to avoid axillary dissection in these node-negative patients without losing the prognostic information derived from a knowledge of nodal status had led to the development of the lymphatic mapping and sentinel node biopsy technique. This procedure is currently performed in many centers (5,6). Although sentinel lymph node (SLN) biopsy has become widely accepted as an alternative to routine axillary dissection for breast cancer, reported FN rates vary widely from \(0\%\) to as high as \(19\%\). The methods used for the identification of metastasis in sentinel nodes in the operating...
room are frozen section examination using hematoxylin–
eosin (H&E) stained tissue, imprint cytology and recently
ultrarapid IHC. The accuracies of H&E frozen sections and
imprint cytology vary from 83–100% and 83–99%, respecti-
vely, and their abilities to detect micrometastasis are limited
(7). Although cytookeratin IHC and RT–PCR using permanent
sections can increase detection rates, they require much time.
Thus, the ultrarapid cytookeratin IHC method for SLN assess-
ment had been widely studied for use in the operating room as
a means of detecting node metastasis with greater sensitivity
and accuracy, and thus of reducing FN rates (8,9).

The aim of the present study was to assess whether the
use of intraoperative ultrarapid cytookeratin IHC enhances
the intraoperative detection of metastasis as compared with
routine frozen H&E stain in breast cancer patients.

MATERIALS AND METHODS

PATIENTS

From September 2004 to November 2005, 79 primary breast
cancer patients with clinically node-negative early breast
carcinoma were treated by breast conserving surgery or mast-
ectomy and SLN biopsy at Samsung Medical Center, Seoul,
Korea. Informed consent was obtained from all patients. SLN
biopsies were performed using radioactive colloid and iso-
sulfan blue dye. Technetium-99m tin-colloid (0.5-mCi) was
injected into subareolar regions 2 h preoperatively; lymph-
oscintigraphy was optionally performed. At the time of sur-
gery, 5 ml of isosulfan blue dye was injected into subareolar
locations, and the areas were massaged gently for ~5 min to
improve lymphatic drainage. After making a small incision at
the axilla, intraoperative SLN identification was performed by
blue dye mapping and by gamma probe detection. We checked
both in vivo and ex vivo radioactivity count. The ex vivo
count was recorded as sentinel node radioactivity. After
excision of the hottest node, the remaining background activity
was checked using hand held gamma probe to find another
sentinel node. An SLN was defined as any blue-stained
node or any node with a radioactivity count larger than
10% of the hottest node. SLNs were examined histologically
in frozen H&E stained sections and by ultrarapid cytookeratin
IHC in all patients. Postoperatively, serial sections, taken at
5 μm intervals, in formalin-fixed and paraffin-embedded
SLN were performed for permanent identification of SLN
metastasis.

HISTOLOGIC EXAMINATION OF SLNs BY RAPID IHC

Metastasis was evaluated in frozen SLNs by H&E staining and
ultrarapid cytookeratin IHC. The lymph nodes were bisected or
trisected in 2 mm interval along their long axis. Two serial
frozen sections at 5 μm interval were cut from each level of
tissue, one for H&E stain and another for cytookeratin immuno-
histochemistry (IHC). After drying the slide at room temper-
ature for 2 min, tissues were fixed for 30 s in cold acetone,
was 96.2%. No false-positive intraoperative diagnosis was identified in both intraoperative studies (specificity 100% and positive predictive value 100%). Ultrarapid IHC enhanced the intraoperative detection of sentinel node metastasis than frozen H&E staining, but no statistically significant differences in sensitivity and accuracy were identified between two intraoperative studies ($P = 0.083$) (Tables 2 and 3).

In 20 patients with metastatic SLNs, 12 were macrometastasis, which was detected in both frozen studies, and all underwent completion axillary dissection simultaneously. In one patient of macrometastasis, additional sentinel node ITC was detected in another sentinel LN. In four cases of micrometastasis, one was detected in rapid IHC only and one case was not detected in both frozen studies. The case that detected intraoperatively only in rapid IHC performed completion axillary dissection in the second operation due to patient condition. This case was rechecked by a pathologist, and the slide of frozen H&E stain did not show any metastatic focus. All patients with sentinel node micrometastasis performed completion axillary dissection. Sentinel node ITC was found in four cases. Frozen H&E stain did not detect any sentinel node ITC but ultrarapid IHC detected two cases of ITC intraoperatively (Figures 1 and 2). In two cases, completion axillary dissection had been performed simultaneously (Table 4).

In patients who underwent completion axillary dissection, the nonsentinel node metastasis was detected only in three patients with sentinel node macrometastasis. The patients with sentinel node micrometastasis and ITC did not have any nonsentinel node metastasis.

**DISCUSSION**

Axillary lymph node status is the most valuable prognostic and decision-making indicator of adjuvant chemo- or radiotherapy in breast cancer. Mastectomy with axillary lymph node

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### Table 1. Characteristics of 79 breast cancer patients that were examined SLN intraoperatively by both frozen H&E staining and ultrarapid IHC

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>$n = 79$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age</td>
<td>48.0 (34–79)</td>
</tr>
<tr>
<td>Histologic tumor size</td>
<td>1.4 (0.2–3.5)</td>
</tr>
<tr>
<td>T staging</td>
<td></td>
</tr>
<tr>
<td>T is</td>
<td>3 (3.8%)</td>
</tr>
<tr>
<td>T1a</td>
<td>10 (12.7%)</td>
</tr>
<tr>
<td>T1b</td>
<td>15 (19.0%)</td>
</tr>
<tr>
<td>T1c</td>
<td>31 (39.2%)</td>
</tr>
<tr>
<td>T2</td>
<td>20 (25.3%)</td>
</tr>
<tr>
<td>Tumor histology</td>
<td></td>
</tr>
<tr>
<td>Ductal</td>
<td>70 (88.6%)</td>
</tr>
<tr>
<td>Lobular</td>
<td>2 (2.5%)</td>
</tr>
<tr>
<td>Others*</td>
<td>7 (8.9%)</td>
</tr>
<tr>
<td>Type of surgery</td>
<td></td>
</tr>
<tr>
<td>Partial mastectomy</td>
<td>59 (74.7%)</td>
</tr>
<tr>
<td>Total mastectomy</td>
<td>20 (25.3%)</td>
</tr>
<tr>
<td>Completion AD</td>
<td></td>
</tr>
<tr>
<td>Not done</td>
<td>54 (68.4%)</td>
</tr>
<tr>
<td>Primary op</td>
<td>22 (27.8%)</td>
</tr>
<tr>
<td>Secondary op</td>
<td>3 (3.8%)</td>
</tr>
</tbody>
</table>

*Mucinous, tubular, medullary and papillary carcinoma.

### Table 2. Comparison of the detection rate of sentinel node metastasis intraoperatively in H&E staining and rapid IHC assay

<table>
<thead>
<tr>
<th></th>
<th>Frozen H&amp;E (%)</th>
<th>Rapid IHC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>59</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>65</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Axillary lymph node status is the most valuable prognostic and decision-making indicator of adjuvant chemo- or radiotherapy in breast cancer. Mastectomy with axillary lymph node

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### Table 3. Comparison of the detection rate of sentinel node metastasis intraoperatively in H&E staining and rapid IHC assay

<table>
<thead>
<tr>
<th></th>
<th>Frozen H&amp;E (%)</th>
<th>Rapid IHC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>70.0</td>
<td>85.0</td>
</tr>
<tr>
<td>Specificity</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Accuracy</td>
<td>92.4</td>
<td>96.2</td>
</tr>
<tr>
<td>PPV</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>NPV</td>
<td>90.8</td>
<td>95.2</td>
</tr>
<tr>
<td>FN rate</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>False-positive rate</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Difference in sensitivity and accuracy not significant, $P = 0.083$, by McNemar’s test. PPV, positive predictive value; NPV, negative predictive value.

### Figure 1.

FN case for micrometastasis at SLN on initial frozen section H&E stain ($\times 200$).
dissection remains the routine surgical treatment for breast cancer. Recently, the incidence of early breast cancer has increased due to advances in detection methodologies and a reduction in the number of axillary node positive cases. In axillary node-negative cases, axillary dissection presents the risk of co-morbidity without therapeutic benefit. The sentinel node biopsy technique is performed to determine axillary lymph node status, and thus to avoid unnecessary axillary dissection.

Sentinel node biopsy was first performed in penile cancer, and the technique has been widely used in melanoma (10,11). Giuliano first used this method in breast cancer in 1994, and now it is used in many centers (12). In terms of the accuracy and FN rates of sentinel node biopsy, the Milan group in 1999 concluded that serial section examinations of sentinel nodes lowered FN rates to 6.5 versus 36% for routine frozen biopsy, and Veronesi found a FN rate of 4.7% for the sentinel node biopsies of 163 clinically node-negative patients (13,14). Sometimes, some cases with a negative intraoperative frozen biopsy result experienced a poor prognosis, and such cases may be associated with micrometastasis in a sentinel node (14). To lower the FN rates of sentinel node biopsy and to detect micrometastasis, several different evaluation methods have been examined, i.e. H&E staining with serial sections, real time RT–PCR and cytokeratin IHC, but these modalities proved time consuming and caused problems of second operation in positive cases. Cytokeratin based ultrarapid IHC was designed to overcome this time limitation and to improved test accuracy, as keratin is not present in normal lymph nodes, and thus was believed to be useful for detecting micrometastasis.

Although many studies have demonstrated the usefulness of rapid IHC (15–17), these findings are compromised by others that did not find any benefit for this method (18). Nevertheless, it is generally agreed that the rapid IHC assay is better than frozen H&E staining in terms of micrometastasis detection.

In the present study, the sensitivity of ultrarapid IHC was higher than that of H&E frozen biopsy (85.0 versus 70.0%), and its accuracy was also higher (96.2 versus 92.4%), though not significant (P-value = 0.083). These results concur with those of others (13).

Micrometastasis in a lymph node is defined as a metastatic tumor size of less than 2.0 mm microscopically. After it was first described by Huvos in 1971, the clinical significance of micrometastasis in SLN has remained unclear (19). Dowlatshahi et al. (20) reported that micrometastasis in a sentinel node might influence patient survival, especially in cases with more than 10 metastatic clusters. Similarly, Rosen et al. (21) reported that disease-free and overall survivals were lower in cases with micrometastasis. In cases of pN0 (i+) by AJCC staging, the clinical significance was controversial. In some results of prospective study, patients with ITC only in the SLN, 14.7% had further axillary involvement (22). The risk of recurrence in these cases remains and intraoperative detection might be helpful in decision making of completion axillary dissection and can avoid second operation.

In the present study, the ultrarapid IHC assay resulted in the detection of one additional case of micrometastasis and three additional ITCs, and two cases of ITC underwent completion axillary dissection simultaneously. The one case showed ITC in four cells and one case was of two sentinel node metastasis, as macrometasis in one and ITC in another lymph node. Sentinel node macrometastasis was detected in frozen H&E stain in all cases and ultrarapid IHC did not have any diagnostic benefit. But the detection of sentinel node micrometastasis and ITC enhanced by ultrarapid IHC. In Korea, the patients sometimes are afraid of a second operation and this method might be helpful in avoiding second operation by detect additional micrometastasis and ITC.

The usefulness of ultrarapid IHC needs to be further studied in a larger cohort.

Table 4. Detection rates of frozen H&E and rapid IHC according to the metastatic tumor size of SLN (n = 20)

<table>
<thead>
<tr>
<th>Class</th>
<th>No. of lymph nodes</th>
<th>Frozen H&amp;E (%)</th>
<th>Rapid IHC (%)</th>
<th>Final results</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITC</td>
<td>0 (0%)</td>
<td>2 (50%)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Micrometastasis</td>
<td>2 (50%)</td>
<td>3 (75%)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Macrometastasis</td>
<td>12 (100%)</td>
<td>12 (100%)*</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14 (70%)</td>
<td>17 (85%)</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

*1 patient of macrometastasis had additional sentinel node ITC detected only in rapid IHC not in frozen H&E staining.
ITCs, isolated tumor cells.

CONCLUSION

To increase the accuracy of the detection of sentinel node metastasis, we used the intraoperative ultrarapid IHC technique in combination with frozen H&E stain biopsy. The ultrarapid IHC method resulted in high sensitivity and accuracy compared with routine frozen H&E stain biopsy.
Intraoperative method used detected one additional micro-metastasis and three ITCs in the operating room, and in two patients ultrarapid IHC helped in avoiding the second operation. In circumstances where the clinical significance of micrometastasis is undetermined, the detection of micrometastasis and ITCs during operation might be helpful in decision making.

References