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 lymphedema, 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%, respec-
tively, and their abilities to detect micrometastasis are limited
(7). Although cytokeratin IHC and RT–PCR using permanent
sections can increase detection rates, they require much time.
Thus, the ultrarapid cytokeratin IHC method for SLN asses-
sment 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 cytokeratin 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-
ery, 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 cytokeratin
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 cytokeratin 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 cytokeratin immuno-
histochemistry (IHC). After drying the slide at room temper-
ature for 2 min, tissues were fixed for 30 s in cold acetone,
dried at room temperature for 1 min, washed in saline and
rinsed in Tris-buffered saline (TBS). Cytokeratin (AE1/AE3,
1:20 Zymed, CA) was used as primary antibody for rapid IHC
and labeled polymer-HRP as a secondary antibody (EnVision
Detection Kit, Dako). After application of primary antibody,
the slide was incubated in the oven for 3 min and 10 s at 37°C.
After washing with TBS, the secondary antibody labeled with
polymer-HRP was applied and incubated in the oven for 3 min
and 10 s at 37°C. After washing, DAB was applied. The stain-
ing took ~20 min. H&E staining was performed and histologic
finding was compared with the result of immunohistochemical
stain. The result was reported to the surgeon in operation
theatre. Remaining specimens were fixed in 4% neutral buf-
fered formalin and paraffinized for permanent study. Twenty
serial sections, taken at 5 μm intervals, in formalin-fixed and
paraffin-embedded SLN were performed for permanent iden-
tification of SLN metastasis. Additional cytokeratin IHC was
performed in one permanent section. In permanent cytokeratin
IHC, the universal LSAB kit (Dako) was used. The other
staining step was same as ultrarapid IHC. Sentinel node micro-
metasis was defined as metastatic foci smaller than 2 mm and
larger than 200 μm and ITCs (isolated tumor cells) was defined
as metastatic foci smaller than 200 μm.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS software,
version 11.5. For each diagnostic method, the sensitivity,
specificity and overall accuracy were calculated. The sensit-
ivities, specificities, accuracies, positive predictive values
and negative predictive values of frozen H&E staining and
ultrarapid IHC were compared using the McNemar’s test.

RESULTS

Patient ages ranged from 34 to 79 years (mean, 48.0 years) and
their mean tumor size was 1.4 cm (0.2–3.5 cm). There were
49 patients with stage I disease, 26 with stage II disease, 3 with
stage 0 disease and 1 with stage III disease. Partial mastectomy
was performed in 59 patients and total mastectomy was in
20 patients. Ductal histology was most prevalent in 70 patients
(Table 1).

A total of 178 SLNs were biopsied in the 79 patients and
mean number of SLNs per patient was 2.3 (1–5). All SLNs
were located in axilla, and the mean time to perform an
intraoperative frozen H&E stain and ultrarapid cytokeratin
IHC was 20 min.

In final result based on permanent serial section and cyto-
eratin IHC, 20 of 79 patients revealed metastasis including
isolated tumor cells. The frozen H&E stain detected 14 cases of
SLN metastasis intraoperatively and ultrarapid cytokeratin
IHC detected 17 cases of metastatic SLNs intraoperatively.
The sensitivities of frozen H&E stain and ultrarapid IHC
were 70.0 and 85.0%, respectively, and the negative predictive
values were 90.8 and 95.2%, respectively. The accuracy of
frozen H&E stain was 92.4% and that of ultrarapid IHC
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 the 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 chemoradiotherapy 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 in cm (range)</td>
<td>1.4 (0.2–3.5)</td>
</tr>
<tr>
<td>T staging</td>
<td></td>
</tr>
<tr>
<td>Tis</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.

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**Table 2.** Comparison between rapid IHC assay and frozen section hematoxylin–eosin (H&E) stain ($n = 79$)

<table>
<thead>
<tr>
<th>Final result</th>
<th>Frozen H&amp;E stain</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>

**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>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.
detected micrometastasis, several different evaluation methods 
(14). To lower the FN rates of sentinel node biopsy and to 
may be associated with micrometastasis in a sentinel node 
biopsy result experienced a poor prognosis, and such cases 
Sometimes, some cases with a negative intraoperative frozen 
biopsies of 163 clinically node-negative patients (13,14). 
and Veronesi found a FN rate of 4.7% for the sentinel node 
lowered FN rates to 6.5 versus 36% for routine frozen biopsy, 
concluded that serial section examinations of sentinel nodes 
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). 
Giulliano 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 
biospies 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 macrometastasis in one and ITC in another lymph 
ode. 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 opera-
tion 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.

CONCLUSION
To increase the accuracy of the detection of sentinel node 
metastasis, we used the intraoperative ultrarapid IHC tech-
nique in combination with frozen H&E stain biopsy. The 
ultrarapid IHC method resulted in high sensitivity and accur-
acy compared with routine frozen H&E stain biopsy. This

<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.
intraoperative method used detected one additional micrometastasis 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