Correlation between Immunohistochemical and Biochemical Estrogen Receptors in the Prognosis of Patients with Breast Cancer

SUMMARY To evaluate the reliability of immunohistochemical estrogen receptor (ER) in the prognosis of patients with breast cancer, 83 primary tumors from patients were studied. Immunohistochemical analysis (IHA) was performed using antibody ER 105 (Dako) together with microwave treatment for antigen retrieval. ER values obtained using the biochemical steroid binding assay (polyethylene glycol method, PEG) were available for comparison. Of all tumors, ER positivity was detected in 44.6% by IHA and 36.1% by PEG method. The concordance between the two methods was 69%. No significant correlation was found between the ER status determined by both methods and clinical stage, tumor size, lymph node status or age of patient at diagnosis. However, we found that the immunohistochemical ER is a superior predictor of early recurrence in patients with primary breast cancer to biochemical ER. The findings in the present study emphasize the clinical benefit of the immunohistochemical ER assay as a measure for prognosis.

MATERIALS AND METHODS

Patients and tissues

This study consisted of 83
primary tumors from women with breast cancer treated at the National Cancer Institute, Bangkok between 1987 and 1989. None of the patients had distant metastasis at the time of operation. All node-positive patients received six cycles of adjuvant chemotherapy containing cyclophosphamide, methotrexate and fluorouracil and local radiation (if the primary tumor was T3 and the patients had inadequate lymph node dissection). For the node-negative patients treatment varied according to T lesion, hormone receptor and age. After surgery, tissue samples were kept frozen at -70°C until use for biochemical determination of ER. A parallel sample was processed using routine techniques for histological examination and immunohistochemical study on paraffin sections. The mean patient follow-up period was more than five years.

Immunohistochemical assay of ER

Three-micrometer thick paraffin embedded sections were deparaffinized in xylene, and rehydrated through alcohol. The sections were washed with phosphate-buffered saline (PBS, pH 7.4) and placed in a plastic coplin jar containing 10 mM citrate buffer (pH 6.0). The jar was heated in a microwave oven (800 w) at high power setting for two 5-minute cycles with an interval of one minute between cycles to check on the water level in the jar. After heating, the coplin jar was removed from the oven and allowed to cool for 15 minutes. The slides were rinsed in PBS and preincubated with 3% normal horse serum in PBS for 30 minutes. The monoclonal antibody to ER 1D5 (Dako, Denmark), diluted 1:100 in PBS, was applied overnight at room temperature. The next day, sections were washed in PBS and incubated for 30 minutes at room temperature with a biotinylated antimouse immunoglobulin (Dako, Denmark) at a dilution of 1:500, then rinsed again with PBS. Antibody binding was visualized by incubation with streptavidin-biotin peroxidase complex (Dako, Denmark) for 1 hour at room temperature. The sections were rinsed in PBS and immersed in a solution of 25 mg diaminobenzidine tetrahydrochloride in 50 ml Tris HCl buffer (pH 7.4) containing 50 μl of 30% hydrogen peroxide and 500 μl of 1 M imidazole for 10 minutes and counterstained with Mayer's hematoxylin for 1-2 minutes. Finally, they were rinsed in tap water, dehydrated in ethanol, cleared in xylene and mounted in permount.

A negative control was obtained by omitting the primary antibody and a section of tumor known to be ER-rich was included as a positive control. Obvious nuclear staining in more than 5% of malignant cells was considered positive.

Biochemical assay of ER

ER content was determined in our routine laboratory using the polyethylene-glycol (PEG) method of Hammond and Braunseberg, previously set up for determination of progesterone receptor content in human endometrium. This technique is similar in principle to the conventional dextran-coated charcoal (DCC) technique. The receptors were identified on the basis of in vitro biochemical technique to measure the specific binding affinity between tritiated steroids and their receptor sites. The free fraction was then separated from the bound by precipitation with the PEG. The affinity and the total binding capacity of the receptors were estimated by Scatchard analysis. The receptor content was expressed as fmol/mg protein. Receptor concentration less than 10 fmol/mg was considered negative.

Statistical analysis

The correlation between the determination of ER by both methods and other clinicopathological features was evaluated by \( \chi^2 \) test. Five-year disease-free survival (DFS) curves were performed by the Kaplan-Meier method \( ^{18} \) and the difference between the curves was assessed using the log rank test. \( ^{19} \)

RESULTS

In this study, the biochemical ER value ranged between 0.00 and 173.2 fmol/mg which yielded 44.6% of the tumors being ER positive. According to the cut-off for positive staining by immunohistochemical assay, 36.1% of the tumors were ER positive. The concordance between the two methods was 69%.

ER determined by either immunohistochemical or biochemical methods was evaluated with respect to different clinicopathological characteristics of the breast cancers. The presence of ER detected by both methods was not significantly associated with stage, tumor size, lymph node status or age of the patients at diagnosis (\( p > 0.05 \)) as shown in Table 1.

To evaluate the predictive value of ER status on five-year recurrence-free survival as shown in Figs. 1 and 2, we found that patients with ER-IHA negative tumors had a probability of recur-
Fig. 1 Five-year disease-free survival (DFS) by immunohistochemical ER status.

Fig. 2 Five-year disease-free survival (DFS) by biochemical ER status.
Table 1 Clinicopathological features of 83 patients in relation to ER status

<table>
<thead>
<tr>
<th>Age at diagnosis (yrs.)</th>
<th>No. of patients</th>
<th>ER-SBA % positive</th>
<th>p</th>
<th>ER-IHA % positive</th>
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<tr>
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<td>15</td>
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SBA = Steroid binding assay  
IHA = Immunohistochemical assay

rence higher than patients with ER-IHA positive tumors with significant difference ($p = 0.0497$) (Fig. 1). According to the biochemical steroid binding assay, no significant difference in five-year recurrence-free survival ($p = 0.8714$) was observed between women with ER negative and ER positive tumors (Fig. 2).

**DISCUSSION**

The PEG method used to biochemically measure the ER in this study was proved to be comparable to the traditional DCC method in determining ER content in breast cancer tissues. The PEG method probably has greater advantages than the DCC method since the polyethylene glycol binds to the hormone receptor complex with greater stability than the dextran coated charcoal. In addition, the DCC method itself is highly sensitive to slight changes in assay conditions.

The immunohistochemical method employed by us utilized a monoclonal antibody (1D5) directed against the N-terminal of the estrogen receptor protein together with microwave treatment for antigen retrieval, which has earlier been demonstrated to be reliable and reproducible.

Rates of concordance between the biochemical and immunohistochemical methods have been reported between 70% and 90%. In our study, a similar percentage of concordance between the two methods was observed.

When considering the relationship between ER status detected by both methods and other clinicopathological characteristics, no correlation was observed in our series. Previous studies on this relationship have yielded variable findings, while some investigators demonstrated a relationship, others did not. These discrepancies probably occurred due to various factors including non-uniform sampling of tumor tissues, differences in receptor determination methods used or differences in the compositions of the patient populations studied.

Previously, ER status determined by biochemical assay had been recognized for a long time as a prognostic factor in primary breast cancer, but in more recent years the longterm predictive value of the ER has been further analyzed and found to disappear when the follow-up of patients is longer than four or five years. Therefore, a longer period of observation is needed to confirm the usefulness demonstrated by the immunohisto-
chemical method. In this study, we found a significantly prognostic effect of the ER detected by immunohistochemistry after following up the patients for more than five years. However, no significant correlation was observed between the biochemical ER status and five-year disease-free survival. Our findings are consistent with those obtained by others, suggesting that immunohistochemical ER is a superior predictor of early recurrence in patients with primary breast cancer than biochemical ER.

To conclude, results obtained with ER analysis in paraffin-embedded sections are promising. In addition, immunohistochemistry performed in paraffin-embedded tissues is simpler and less expensive than analysis using biochemical methods. Moreover, the number of resected breast cancers that have insufficient tissue for biochemical determination of hormone receptors is currently increasing due to smaller tumors obtained when the disease can be detected earlier. Thus, the immunohistochemical method is appropriate to replace biochemical assay for routine purposes.

ACKNOWLEDGEMENT

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REFERENCES