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Immunohistochemical Detection of Estrogen and Progesterone Receptors in Primary Breast Cancer

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SUMMARY **To evaluate the reliability of the immunohistochemical assay for estrogen receptor (ER) and progesterone receptor (PR) in the prognosis of patients with breast cancer, 83 primary tumors from the patients were studied. Immunohistochemical analysis was performed using antibody ER 1D5 for ER determination and antibody PR-ICA for PR determination. Of all tumors, ER and PR positivities were detected in 36.1% and 45.8% respectively. There was no significant relationship between ER, PR and age of the patients, tumor size or number of involved nodes. However, we found that only the immunohistochemical ER was a predictor of early recurrence in patients with primary breast cancer. In addition, there was no additive effect in recurrence-free survival when both receptor expressions were combined.**

Steroid hormone receptor status is an important predictor of prognosis and response to endocrine therapy in patients with breast cancer. Most data on the prognostic implication of estrogen and progesterone receptor (ER and PR) determinations have been generated with biochemical assays employing the dextran-coated charcoal method. More recently, immunohistochemical assays have gained in popularity due to their lower costs and the lower requirements for sample size as well as the possibility to perform the assay on routine formalin-fixed, paraffin-embedded tissues.

In general, discrepancies between receptor measurements in tumor homogenates and the immunohistochemical demonstration are due to the different concentration of tumor cells in several histological types of breast carcinoma,[1] to the heterogeneity of receptor expression[2] and to a possible contamination of the biochemical samples by non-cancerous breast tissue.[3] For these reasons the immunohistochemical method is in theory superior to biochemical determination because various sources of error are eliminated.

In our previous study,[4] we found that determination of the receptors by both methods yielded 74.5% of concordant findings for the ER and 74.4% for the PR. Rates of concordance between the biochemical and immunohistochemical methods have been reported between 70% and 90%.[5-9]

The present study aimed to detect the presence of ER and PR in routine formalin-fixed, paraffin-embedded tissue specimens of breast carcinomas using an immunohistochemical method and assessed the correlation of ER and PR expression with clinicopathological features including five-year recurrence-free survival. Moreover, we also examined the effects of the two receptors coexpression on five-year recurrence-free survival.

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MATERIALS AND METHODS

Patients and tissues

This study included 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 is 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 routine 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 method

Antibodies used to detect ER and PR in this study were anti-ER mouse monoclonal antibody (ER1D5; Dako, Denmark) and anti-PR rat monoclonal antibody (PR-ICA; Abbott Laboratories,USA) respectively. 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 the high power setting for two 5-minute cycles with an interval of one min 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 antibodies to ER and PR diluted 1:100 and 1:50, respectively, in PBS were 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) for ER and a biotinylated antirat immunoglobulin (Dako, Denmark) for PR at dilutions 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.

Negative controls were obtained by omitting the primary antibodies and sections of tumor known to be ER and PR rich were included as positive controls. Obvious nuclear staining in more than 5% of malignant cells were considered positive.

Statistical analysis

The correlation between the determination of ER and PR and other clinicopathological features were evaluated by χ^2 test. Five-year disease-free survival (DFS) curves were performed by the Kaplan-Meier method [10] and the differences between the curves were assessed using the log rank test.[11]

RESULTS

According to the cut-off for positive immunostaining, 30 (36.1%) breast tumors were positive for ER and 38 (45.8%) were positive for PR. The association between ER, PR and other clinicopathological features is summarised in Table 1. The presence of ER or PR was not significantly associated with tumor size, lymph node status or age of patients at diagnosis ($P > 0.05$).

To evaluate the predictive value of ER and PR on five-year disease-free survival (DFS) as shown in Figs. 1 and 2, we found that patients with ER or PR negative tumors had a probability of recurrence higher than patients with ER or PR positive tumors. However, the statistically significant difference was found only in ER evaluation ($P = 0.0497$ for ER, $P = 0.0581$ for PR). Five-year disease-free survival curves were also plotted for the combination of ER and PR expression as demonstrated in Fig. 3. Patients whose tumors were either ER or PR positive showed a better survival than patients with one type of receptor positivity. Only patients negative for both receptors had the lowest survival probability. This effect was, however, not statistically significant ($P = 0.0744$).

Table 1 Clinicopathological characteristics of 83 patients in relation to ER- and PR-status

Characteristics	No. of patients	ER % positive	P	PR % positive	P
Age at diagnosis (yrs)					
≤ 50	51	29.4		51.0	
> 50	32	46.9	0.1069	37.5	0.2302
Tumor size (cm)					
≤ 3	65	35.4		50.8	
> 3	12	50.0		33.3	
Unknown	6	0	0.3677	0	0.1782
No. positive axillary nodes					
0	37	45.9		40.5	
1-3	20	25.0		55.0	
> 3	26	30.8	0.2297	46.2	0.5782

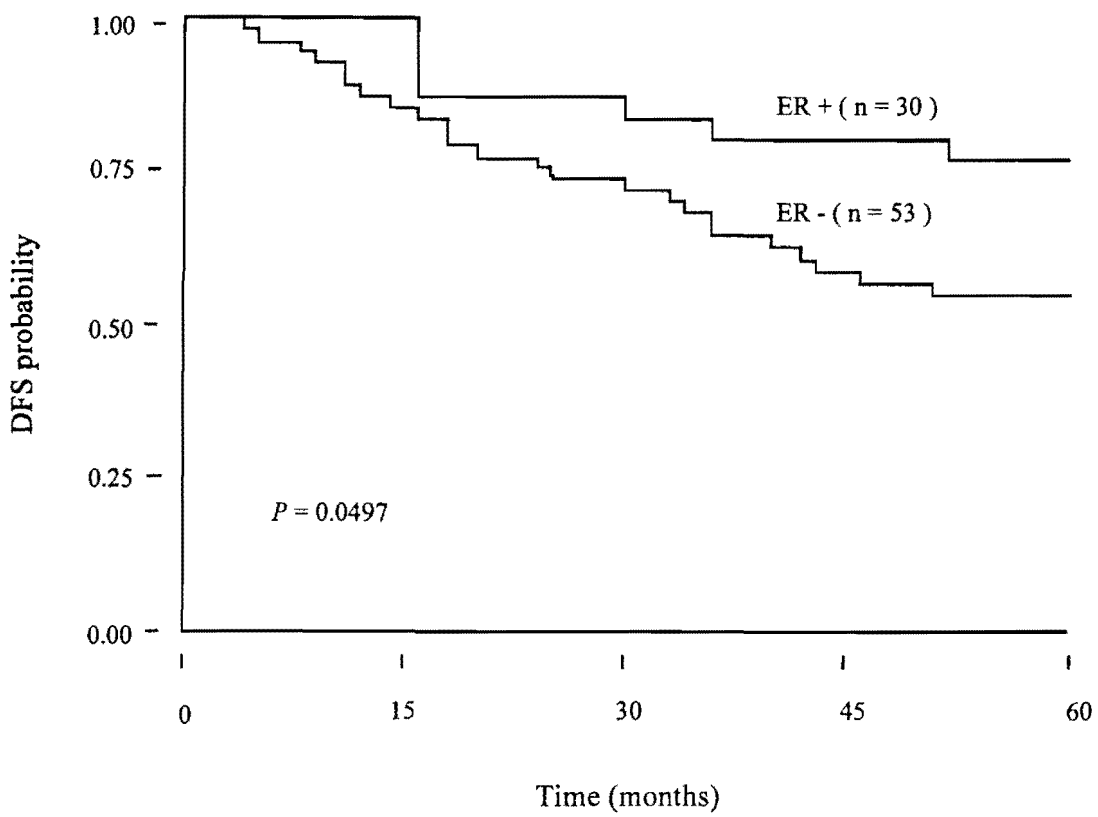

Fig. 1 Correlation between five-year disease-free survival and ER status.

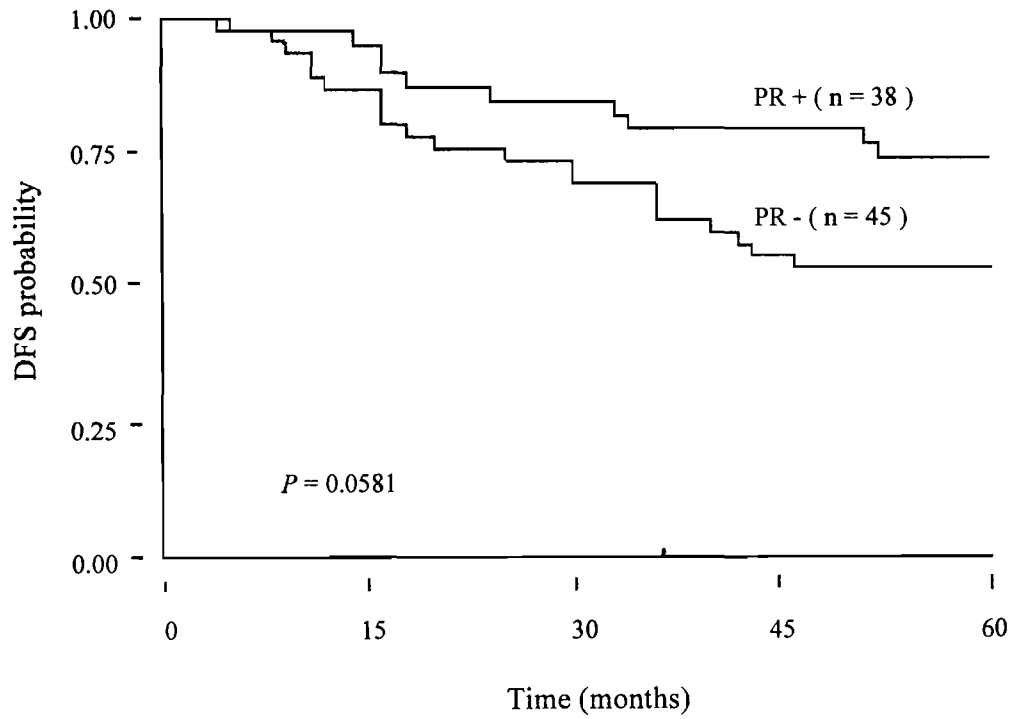

Fig. 2 Correlation between five-year disease-free survival and PR status.

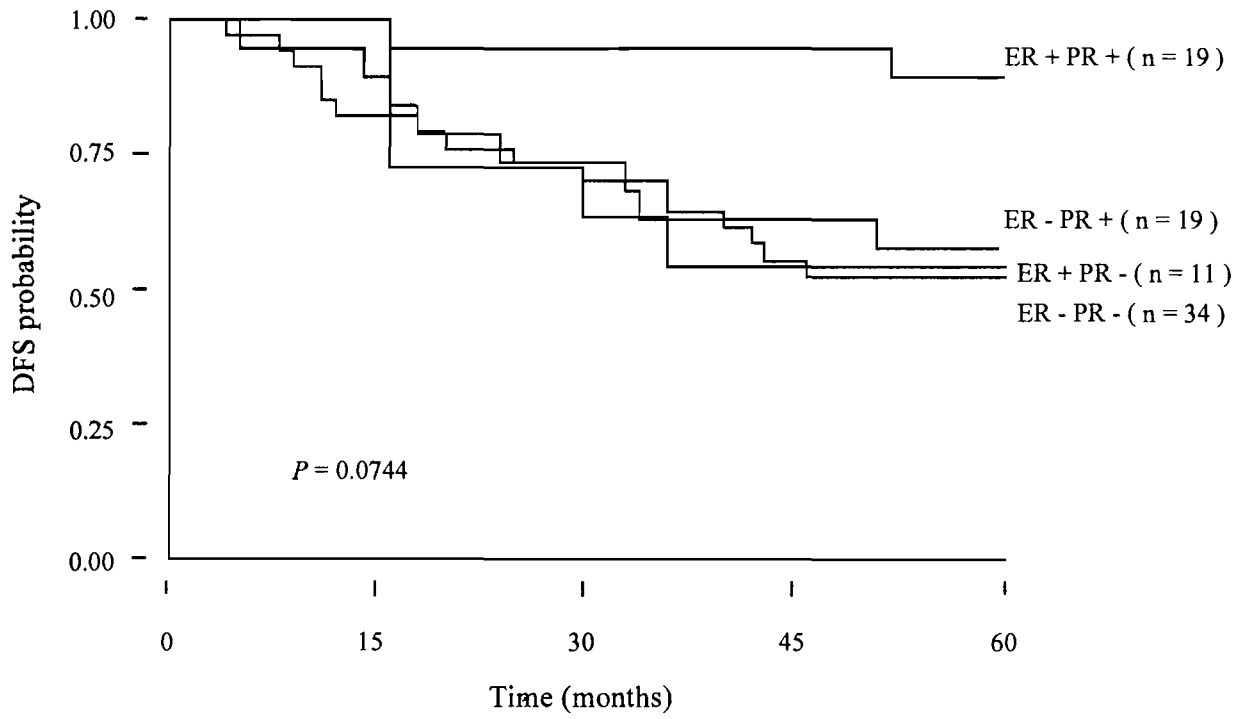

Fig. 3 Five-year disease-free survival by both ER and PR immunostaining.

DISCUSSION

In this study, the monoclonal antibodies used to detect ER and PR together with microwave treatment for antigen retrieval has earlier been demonstrated to be reliable and reproducible.[12-14]

A wide range of immunohistochemical investigations has been reported on positivities of ER and PR in breast cancer. Using different monoclonal antibodies and staining conditions, ER positivity has been examined between 50% and 80%,[6,9,12] while PR positivity has been detected between 40% and 70%.[6,9,12] In the present study, positivities of ER and PR were found in 36.1% and 45.8% respectively. The percentage of ER positivity in our study is lower than that in other reports, probably due to the difference in numbers of early cancers examined or the difference in antibodies used.

The lack of relationship between ER, PR and clinicopathological features was noted in our study. The statistically significant correlation between menopausal status or age and immunohistochemical ER is a matter of controversy in most reports,[5,6,15,16] while PR expression was related to age only in a few studies.[5,6] Several authors found a positive association between ER and PR status and tumor size,[15,17] while others[18,19] agree with our results.

Our data confirm that the number of positive lymph nodes is not influenced by the receptor status of primary tumors, as already stated in various other reports.[6,17,20]

The prognostic importance of ER and PR has been studied in many series, but results are controversial. These varying results probably occurred due to several factors, including the heterogeneity of the patient populations studied or the therapeutic modalities used.

A previous study found that women with immunohistochemical ER-positive or PR-positive carcinomas had a more favorable survival than women with hormone receptor-negative carcinomas, being statistically significant effects.[21] In our study, we also found the same prognostic effects in the determination of ER and PR, but a statistically significant effect was found only in ER evaluation. Our findings are consistent with the results in another study, which revealed a higher prognostic value of the ER status.[21]

When ER and PR were combined, no statistically significant influence on survival was noted in the present study. This is in disagreement with some other reports.[9,21] In those reports, patients with both negative immunohistochemical ER and PR showed a better survival than patients with only one negative receptor or both positive receptors, being statistically significant effects. The reasons for the discrepancies between our results and theirs can be due to various factors including the difference in antibodies used, the heterogeneity of the population studied and the systemic adjuvant therapy.

Our findings confirm that high rates of recurrences occur in patients whose tumors are ER negative. In addition, there was no additive effect in recurrence-free survival when both receptors expression were combined.

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