Comparison of Evaluations for Hormone Receptors in Breast Carcinoma Using Two Manual and Three Automated Immunohistochemical Assays

Koji Arihiro, MD, PhD,1 Shinobu Umemura, MD, PhD,2 Masafumi Kurosumi, MD, PhD,3 Takuya Moriya, MD, PhD,4 Tetsunari Oyama, MD, PhD,5 Hiroko Yamashita, MD, PhD,6 Yoshihisa Umekita, MD, PhD,7 Yoshifumi Komoike, MD, PhD,8 Chikako Shimizu, MD, PhD,9 Hisaki Fukushima, MD, PhD,10 Hiroshi Kajiwara, MD, PhD,2 and Futoshi Akiyama, MD, PhD11

Key Words: Hormone receptors; Estrogen receptor; Progesterone receptor; Immunohistochemistry; Automated staining; Scoring system

DOI: 10.1309/4D1A04NCDK96WFY7

Abstract

The aims of this study were to compare the quality of immunohistochemical assays of estrogen receptor (ER) and progesterone receptor (PR) and to compare the intermethod variability of assays from different manufacturers. Immunohistochemical staining was entrusted to the following laboratories in Japan: Kyowa Medex, dealing with the products of BioGenex (Mishima, Shizuoka), DAKO Japan (Kyoto), and Ventana Japan (Yokohama). All slides were semiquantitatively evaluated according to the Allred score. Intermethod variability showed fair to moderate interrater $\kappa$ values for ER and PR (for total score, ER, $\kappa = 0.34$; PR, $\kappa = 0.45$). Another scoring system was also applied in which, irrespective of the intensity of nuclear staining, the proportion of cells stained in each specimen was recorded as 0; less than 1%; 1% or more and less than 10%; or 10% or more. Intermethod variability showed substantial interrater $\kappa$ values for ER and PR (according to percentage of positive cells, ER, $\kappa = 0.67$; PR, $\kappa = 0.72$). Concerning intermethod consistency, the scoring system based on the percentage of positive cells was advantageous over other scoring systems.

Recently, a refined assessment of hormone receptors in breast carcinoma has become necessary to select therapeutic agents according to the recommendations and guidelines for postoperative adjuvant systemic therapy of early breast cancer proposed by the International Consensus Panel during the St Gallen Conference in 2005.1 The guidelines proposed 3 disease responsiveness categories: endocrine responsive, endocrine response uncertain, and endocrine nonresponsive. In particular, endocrine response uncertain was defined as some expression of steroid hormone receptors quantitatively low (usually considered as <10% of cells positive) or qualitatively insufficient to indicate a substantial chance for response to endocrine therapies alone, suggesting potential resistance to particular endocrine therapies and the need for chemotherapy, but the exact cutoff between endocrine responsive and endocrine response uncertain has not been determined.

As to the method for the detection and quantification of estrogen receptor (ER) and progesterone receptor (PR), immunohistochemical methods have been preferred because of their relative simplicity, low cost, speed of performance, application to small samples, precise identification of reactive elements, simple methods of fixation and storage, ability to be applied to archival material,2 and better ability to predict response to adjuvant endocrine therapy owing to validation studies for ER3 and PR.4 A panel of experts from the College of American Pathologists, the American Society of Clinical Oncology, and the National Institutes of Health recommended assessing ER and PR by immunohistochemical methods rather than by ligand-binding assay (LBA) but expressed reservations about assessing ER and PR by immunohistochemical methods because of the lack of a standardized assay.5-7 From a technical viewpoint, although there have been
many studies using the manual immunohistochemical method on formalin-fixed, paraffin-embedded tissue specimens,\textsuperscript{2,4,8-24} a few newly developed immunohistochemical methods using automated staining devices to do a standardized assay have been published.\textsuperscript{3,25-30} However, there are unresolved problems regarding tissue preparation, including fixation and antigen retrieval, the various types of antibodies, detection reagents, and methods of interpreting the results.

As for the evaluation of immunohistochemical results, some scoring systems, including the Allred score, combine intensity and the number of positive cells in different ways to calculate the score.\textsuperscript{3,4,26} On the other hand, irrespective of the intensity of reactive cells, there have been various cutoffs of the percentage of positive cells ranging from any positive cells or 1%\textsuperscript{14,8,31} through 5%\textsuperscript{24} and 10%\textsuperscript{11,30} to 20%.\textsuperscript{16,29} Although a few validated immunohistochemical methods have been published for ER and PR,\textsuperscript{3,4,32-35} 10% or more of positive nuclei was recommended as a reasonable threshold by the International Consensus Panel at the St Gallen Conference.\textsuperscript{1} Accordingly, up to the present, there has been uncertainty about the correct cutoff for immunohistochemical detection of ER and PR in breast carcinomas.

In Japan, to standardize the immunohistochemical method and to decide on a scoring system, the Japanese Society of Breast Cancer organized a task force to produce an "Adequate evaluation for immunohistochemical evaluation in routine practice for breast cancer." The immunohistochemical methods approved by the Japanese Ministry of Health, Labor and Welfare to assess hormone receptor status for determining suitability for endocrine treatment (manual and automated immunohistochemical staining by DAKO, Glostrup, Denmark, and BioGenex, San Ramon, CA, and automated immunohistochemical staining by Ventana Medical Systems, Tucson, AZ) each use different ways of retrieving antigens and different types of antibodies or detection reagents, whereas until now, no study has directly compared these immunohistochemical methods. The purpose of the present study was to assess the quality of the different immunohistochemical assays carried out by different laboratories and to assess intermethod variability. An additional aim was to find techniques that could be suggested as reliable assays for ER and PR determination.

### Materials and Methods

#### Samples

The study included 89 consecutive cases of invasive ductal carcinoma of the breast that had been surgically resected before July 2003, selected from the files of the Department of Pathology, Saitama Cancer Center, Saitama, Japan. H&E-stained slides of each case were reviewed, and the presence of invasive carcinoma and adjacent nonneoplastic breast tissue was confirmed in all cases. The histologic type of each tumor was invasive ductal carcinoma, not otherwise specified, and no other histologic types, such as lobular, tubular, or mucinous, were included.

#### Immunohistochemical Assays for ER and PR

From formalin-fixed, paraffin-embedded tissue samples, five 4-µm-thick sections were serially cut and mounted on pre-coated slides. To obtain immunohistochemical slides of the highest quality, immunohistochemical staining was entrusted to the laboratory of each manufacturer in Japan, and unstained slides were sent to the following laboratories in Japan: Kyowa Medex, dealing with the products of BioGenex (Mishima, Shizuoka), DAKO Japan (Kyoto), and Ventana Japan (Yokohama). The immunohistochemical staining procedure was thus performed not only according to a previous report\textsuperscript{36} but also according to the in-house protocol of each manufacturer for manual or automated staining Table 1.

<table>
<thead>
<tr>
<th>Protocols for Immunohistochemical Analysis by the BioGenex, DAKO, and Ventana Methods*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BioGenex</strong></td>
</tr>
<tr>
<td>Antigen retrieval</td>
</tr>
<tr>
<td>Device</td>
</tr>
<tr>
<td>Incubation time, min</td>
</tr>
<tr>
<td>Incubation temperature, °C</td>
</tr>
<tr>
<td>Primary antibody</td>
</tr>
<tr>
<td>ER</td>
</tr>
<tr>
<td>PR</td>
</tr>
<tr>
<td>Incubation time, min</td>
</tr>
<tr>
<td>Incubation temperature</td>
</tr>
<tr>
<td>Enhancement method</td>
</tr>
<tr>
<td>Manual staining</td>
</tr>
<tr>
<td>Automated staining</td>
</tr>
</tbody>
</table>

ER, estrogen receptor; LSAB, labeled streptavidin-biotinylated antibody; PR, progesterone receptor.

* BioGenex, San Ramon, CA; DAKO, Glostrup, Denmark; and Ventana, Tucson, AZ.
For immunohistochemical assay by the BioGenex system using manual immunostaining and an automated immunostainer OptiMax Plus (BioGenex, San Ramon, CA), an anti-ER mouse monoclonal antibody (mAb), ER88 (BioGenex), and an anti-PR mAb, PR88 (BioGenex), were used. For antigen retrieval, the sections were manually immersed in solution, Antigen retrieval citra plus (BioGenex), and heated in an autoclave at 121°C for 10 minutes. Endogenous peroxidase activity was quenched by immersion in 3% hydrogen peroxide for 10 minutes. The tissue sections were incubated with primary antibody ER88 for 60 minutes or with primary antibody PR88 for 30 minutes at room temperature. Immunoperoxidase staining was performed using the labeled streptavidin biotin (LSAB) staining kit according to the manufacturer’s instructions (BioGenex), and sections were counterstained with hematoxylin.

For immunohistochemical assay by the DAKO system using manual immunostaining and the DAKO Autostainer (Glostrup, Denmark), an anti-ER mAb, 1D5 (DAKO), and an anti-PR mAb, PgR636 (DAKO), were used. For antigen retrieval, the sections were manually immersed in Target retrieval solution, high pH (DAKO), and heated in a water bath at 95°C to 99°C for 40 minutes. Endogenous peroxidase activity was quenched by immersion in 3% hydrogen peroxide for 5 minutes. The tissue sections were incubated with primary antibody for 30 minutes at room temperature. Immunoperoxidase staining was performed using the LSAB system, EnVision+ kit/HRP, according to the manufacturer’s instructions (DAKO), and sections were counterstained with hematoxylin.

For immunohistochemical assay by the Ventana system using the Ventana HX system BenchMark (Ventana Medical Systems, Tucson, AZ), an anti-ER mAb, 6F11 (Ventana), and an anti-PR mAb, 16 (Ventana), were used. All procedures were performed automatically in the BenchMark. For antigen retrieval, retrieval solution (Ventana) was automatically poured on the sections, and they were then heated on a slide heater at 100°C for 60 minutes. Endogenous peroxidase activity was quenched by immersion in 3% hydrogen peroxide for 4 minutes. The tissue sections were incubated with primary antibody for 32 minutes at 42°C. Immunoperoxidase staining was performed using the LSAB system, NeuVision, according to the manufacturer’s instructions (Ventana), and sections were counterstained with hematoxylin.

Scoring System

First, the presence or absence of staining of the nuclei of nonneoplastic ducts and acini in adjacent tissue was observed, and this was used as an internal control sample. The site for evaluation was not limited to the invasive area but incorporated the whole of the lesion. We used 2 scoring systems to evaluate immunohistochemical findings, our own score and the Allred score, because the cutoff value of the Allred score is clinically validated in some studies. The Allred score consists of a proportion score (PS; 0, none; 1, <1/100; 2, 1/100 to 1/10; 3, 1/10 to 1/3; 4, 1/3 to 2/3; and 5, >2/3) and an intensity score (IS; 0, negative; 1, weak nuclear staining, faintly perceptible at high-power magnification; 2, intermediate nuclear staining; and 3, nuclei displaying strong staining with the appearance of an ink dot at low-power magnification). The PS and IS were added to obtain a total score (TS; range, 0, 2-8).

We also set up another scoring system in which, irrespective of the intensity of stained nuclei, the proportion of cells stained in each specimen was recorded as 0, less than 1%, 1% or more but less than 10%, or 10% or more; these have been advocated and used as cutoffs in previous reports.

Pathologists were trained in the use of the immunohistochemical scoring system by simultaneous assessment of about 90 breast carcinoma tissue samples that were immunostained for ER and PR. A consensus was obtained on discordant samples after discussion using a microscope projector. After that, all study specimens were scored by 1 pathologist (K.A.) who was unaware of patient characteristics.

Statistical Analysis

The relationship between the categorical variables (ER and PR) was analyzed by using the χ² test. The multirater κ was used as a measure of variability for intensity, percentages of immunoreactive cells, and resulting score. The κ values were calculated and interpreted according to previous reports.

Results

Distribution

The frequency distributions of ER immunohistochemical results based on the Allred score are shown in Figure 11, Figure 12, Figure 13, Table 21, Table 31, and Table 41. The distributions of PS, IS, and TS according to the Allred score were significantly different (Tables 2, 3, and 4, respectively), suggesting that the intensity and proportion were higher in DAKO (manual and automated) and Ventana (automated) than in BioGenex (automated). On the other hand, the distribution of the percentage of ER+ cells was not significantly different in Figure 41 and Table 51.

The frequency distribution of PR immunohistochemical results based on the Allred score is shown in Figures 1, 2, and 3 and Tables 2, 3, and 4. Although the distribution of IS according to the Allred score was significantly different...
Figure 1 presents the distribution of the proportion scores for estrogen receptor (A) and progesterone receptor (B) detected by 5 methods according to the Allred score. Although the distribution of proportion scores for estrogen receptor was significantly different in each method ($P < .020; \chi^2$), the distribution of proportion scores for progesterone receptor was not ($P = .128; \chi^2$). For proprietary information, see Table 1.

Figure 2 shows the distribution of the intensity scores for estrogen receptor (A) and progesterone receptor (B) detected by 5 methods according to the Allred score. The intensity scores for estrogen receptor and progesterone receptor were significantly different in each method ($P < .0001; \chi^2$). For proprietary information, see Table 1.
Distribution of the total scores for estrogen receptor (A) and progesterone receptor (B) detected by 5 methods according to the Allred score. Although the distribution of the total scores for estrogen receptor was significantly different in each method ($P = .0002; \chi^2$), the distribution of total scores for progesterone receptor was not ($P = .1868; \chi^2$). For proprietary information, see Table 1.

Among methods (Table 3), the distribution of PS, TS, and percentage of PR+ cells were not significantly different. The intensity for PR immunohistochemical results was significantly lower in the DAKO manual method than in the other 4 methods.

As for the relationship between PS and IS according to the Allred score, the increase of PS was significantly correlated with the increase of IS in all 5 methods ($P < .0001$).

Concordance
Overall $\kappa$ values are summarized in Table 6. Intermethod variability evaluated according to TS, PS, and IS by the Allred score and the percentage of positive cells showed fair to substantial $\kappa$ values for the ER and PR scores, whereas the PR was better than the ER. Concerning intermethod consistency, the scoring system based on the percentage of positive cells was advantageous over other scoring, including TS, PS, and IS by the Allred score.

<table>
<thead>
<tr>
<th>Staining Method</th>
<th>ER†</th>
<th>PR‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>BioGenex</td>
<td>18 (20)</td>
<td>9 (10)</td>
</tr>
<tr>
<td>Manual</td>
<td>16 (18)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Automated</td>
<td>21 (24)</td>
<td>6 (7)</td>
</tr>
<tr>
<td>DAKO</td>
<td>16 (18)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Manual</td>
<td>15 (17)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Automated</td>
<td>16 (18)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Ventana</td>
<td>15 (17)</td>
<td>3 (3)</td>
</tr>
</tbody>
</table>

ER, estrogen receptor; PR, progesterone receptor.
† Data are given as number (percentage). For proprietary information, see Table 1.
‡ $P = .020$, for the distribution of proportion scores.
§ $P = .1280$, for the distribution of proportion scores.
In the present study, the distribution of TSs, ISs, and PSs of ER, according to the Allred score, was significantly different ($P = .0002$, $P < .0001$, and $P = .020$, respectively), suggesting that ISs and PSs of ER by the manual and automated staining methods of BioGenex were smaller than those obtained by other methods. The distribution of ISs was more different from that of PSs, suggesting that the sensitivity of ER88, the efficiency of antigen retrieval, and the amplification of intensity by the manual and automated staining methods of BioGenex may be smaller than those obtained by other methods.

As for ER, the bimodal distribution of the ER score by DAKO manual staining using antibody 1D5 was pointed out, ie, in more than 94% or 99% of cases, the tumors were completely ER– or unequivocally ER+. These results suggest that the DAKO immunohistochemical assay is too sensitive and perhaps not sufficiently specific. On the other hand, one study has shown that there is considerable intermethod variability in the identification of tumors with lower levels of ER expression. Our results were similar to the results given in the latter study. Accordingly, it might be difficult to compare our results with those of other institutions and generalize the results. Although Fisher et al. showed that the any-or-none immunohistochemical method for scoring ER seemed valid as a prognostic indicator of overall survival in patients with positive lymph nodes, they did not examine tumor samples from patients with negative lymph nodes. On the other hand, we could not examine the validity of each cutoff value based on data for overall survival, disease-free survival, or recurrence-free survival. Accordingly, it might be difficult simply to compare our results with those of Fisher et al. In any case, further validation is necessary to evaluate an optimal cutoff value in our results.

For PR, although the distribution of ISs according to the Allred score was significantly different (Table 3), the distributions of TSs and PSs for PR were not significantly different from each other (Tables 2 and 4), suggesting that the ISs of PR by DAKO manual staining were smaller than those by other methods.
To our knowledge, only 1 study assessed PR status according to the Allred score by immunohistochemical staining using antibody 1294 (DAKO), showing that 43% had a TS of 0, 1.3% had a TS of 2, 2.6% had a TS of 8, and the rest of TSs were approximately uniformly distributed, ranging from 9% to 12%. Although these results differ from ours, it is difficult for us to explain the reason. Some reasons why comprehensive studies of PR by immunohistochemical assays have lagged behind those of ER have been pointed out: until now, many clinicians have depended on ER status alone to select patients for hormonal therapy because PR status alone has been found to be a weaker prognostic and predictive factor than ER in some studies using ligand-binding assay. Because lack of PR expression, irrespective of ER expression, is regarded as

### Table 5
Distribution of the Percentage of Cells Showing Nuclear Staining for ER and PR by Five Methods in 89 Samples

<table>
<thead>
<tr>
<th>Staining Method</th>
<th>ER</th>
<th>PR</th>
<th>ER</th>
<th>PR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>&lt;1%</td>
<td>1% -&lt;10%</td>
<td>≥10%</td>
</tr>
<tr>
<td>BioGenex Manual</td>
<td>18 (20)</td>
<td>9 (10)</td>
<td>8 (9)</td>
<td>54 (61)</td>
</tr>
<tr>
<td>Automated</td>
<td>21 (24)</td>
<td>6 (7)</td>
<td>13 (15)</td>
<td>49 (55)</td>
</tr>
<tr>
<td>DAKO Manual</td>
<td>16 (18)</td>
<td>4 (4)</td>
<td>3 (3)</td>
<td>66 (74)</td>
</tr>
<tr>
<td>Automated</td>
<td>16 (18)</td>
<td>3 (3)</td>
<td>4 (4)</td>
<td>66 (74)</td>
</tr>
<tr>
<td>Ventana Manual</td>
<td>15 (17)</td>
<td>3 (3)</td>
<td>5 (6)</td>
<td>66 (74)</td>
</tr>
</tbody>
</table>

ER, estrogen receptor; PR, progesterone receptor.

* Data are given as number (percentage). For proprietary information, see Table 1.

† P = .533, for the distribution of the percentage of positive cells.

‡ P = .918, for the distribution of the percentage of positive cells.
one of the features indicative of uncertainty of endocrine responsiveness, suggesting potential resistance to endocrine therapies, the significance of appropriate evaluation of PR is the more important.

With regard to cutoff value, according to the Allred score, tumors are defined as ER+ or PR+ if their TS is greater than 2, corresponding to as few as 1% to 10% of weakly positive cells on the basis of validation studies. On the other hand, it seems that most laboratories arbitrarily use 10% as a cutoff to define ER and PR positivity. When we applied 10% or more as a cutoff to define ER positivity according to the International Consensus Panel, the percentage of ER+ cases ranged from 55% to 74% in the 5 methods, and there were no significant differences in the distribution of ER+ cases (Table 5). In previous reports using the same cutoff value, ER+ cases were reported to be 76.3%, 73.5% by 1D5 (DAKO), and 74% by 6F11 (Novocastra). Our results concerning ER are thought to be similar to those of previous reports. Although the distribution of PSs of ER, according to the Allred score, was significantly different (Table 2), the distribution of the percentage of cells showing nuclear staining for ER was not significantly different (Table 5), suggesting that the scoring system based on the percentage of positive cells was advantageous over other scoring, including TSs, PSs, and ISs of ER by the Allred score.

The percentage of PR+ cases ranged from 51% to 55% in the 5 methods, and there were no significant differences in the distribution of PR+ cases (Table 5). In previous reports using the same cutoff value, PR+ cases were reported to be 53.9% by 1A6 (Novocastra). Our results concerning PR are thought to be similar to those of the previous report.

Intermethod variability of ER and PR status according to the Allred score showed a fair to moderate κ value, and that according to percentage of positive cells showed a substantial κ value, suggesting that evaluation based on the percentage of positive cells shows better agreement than that based on the Allred score. In a previous report concerning comparison of immunohistochemical methods in 31 laboratories (14 using manual staining, 11 using a Ventana autostainer, and 6 using a DAKO autostainer), κ values for the ISs, PSs, and TSs of ER according to the Allred score by DAKO were reported to be the best values among them: 0.62, 0.55, and 0.45, respectively. On the other hand, κ values for ISs, PSs, and TSs of PR according to the Allred score by Ventana were reported to be the best values among them: 0.39, 0.51, and 0.36, respectively. However, we cannot simply make a comparison with κ values based on the Allred score or percentage of positive cells in other studies because there have been no published reports similar to our study.

It is more important to use an assay that is unlikely to produce false-negative results than to be concerned about shifting weakly or moderately positive results toward the higher end of ER positivity. Accordingly, the most important consideration in the use of immunohistochemical assays for hormone receptor status should be the correct identification of tumors with even low levels of hormone receptor expression as being hormone receptor–positive.

As for fixation time, in general, it is noted that immunohistochemical results for ER are highly dependent on the time of tissue fixation. Overfixation leads to antigen masking, probably through aldehydic linkage between proteins and formalin; on the other hand, underfixation is shown to have a more detrimental effect on immunohistochemical results than overfixation. According to a previous report, the minimum fixation time for optimal immunohistochemical results of ER is 6 to 8 hours, regardless of specimen type or size.

Storage of unstained paraffin sections at room temperature resulted in a variable but progressive decrease in expression of several tissue antigens. Although the loss in antigenicity is proportional to the length of storage, the effect was reversible when superantibody concentrations were used. In addition, long-term storage of sections on slides before reaction has been blamed for false-negative results. In the present study, the fixation time of each tumor was not so different, and unstained paraffin sections were not left at room temperature, so immunohistochemical results should not be affected by fixation time or loss in antigenicity. In general, the efficiency of the antigen-retrieval step is identified as the single most important contributory factor influencing the overall reproducibility of the assay. Irrespective of that, immunohistochemical laboratories could achieve a technical improvement by combining different antigen-retrieval methods and detection systems. Lack of technical standardization and intermethod reproducibility might also influence the predictability of the immunohistochemical assay’s meaningful clinical end points.

In the present study, we used 3 types of anti-ER antibody, available for paraffin-embedded sections, 1D5, 6F11, and ER88. Of these antibodies, 1D5 has been used in the most published reports concerning breast carcinoma, and 6F11 in some reports and ER88 in 2 reports. In addition, other clones, 18-0174 and M7047, were available for paraffin-embedded sections, 1D5, 6F11, and ER88.
have been used. Although the epitope for the 1D5 has been assessed by identification of binding peptides, no other assays for the epitope for other antibodies and no information about them have been shown. On the other hand, we used 3 types of anti-PR antibody available for paraffin-embedded sections, PgR636, 16, and PR88. Of these antibodies, although use of PR88 has been described in 2 reports, use of the other 2 has not been described in a published report. 1A6 has been described in the most published reports concerning breast carcinoma, and other clones, PR-2C5,19 1294,35 and PR316,21 have been described. Accordingly, it is difficult for us to compare directly the characteristics of the 3 types of antibody for ER and PR used in the present study.

There are no commercially available quantitative ER and PR control samples. Although ER quantification has important therapeutic implications, intermethod reproducibility is shown to be suboptimal. One of the reasons for this problem is thought to be the inability of laboratories to quantitatively detect out-of-range assay conditions, and, realistically, there are no internationally agreed-on reference materials. Accordingly, immunohistochemical control has to be established using immortalized cell lines, or some specific peptides, and so on instead of tissue sections.

In previous reports, some computerized image analyses were tried for estimating intensity and percentage of positive cells. Accordingly, in the future, to standardize the scoring of ER and PR in immunohistochemical specimens, an appropriate image analysis for quantitative assessments is necessary.

Until now, there have been many published studies that have assessed ER and PR by immunohistochemical analysis. However, these studies have used many different antibodies of varying sensitivity and specificity, a variety of other reagents, different scoring systems, and often arbitrary definitions of ER and PR cutoffs to define positivity, making it extremely difficult to compare results. Nevertheless, as far as intermethod consistency is concerned, according to κ values, the scoring system based on the percentage of positive cells was advantageous over other scoring, including TSs, PSs, and ISs by the Allred score. However, the final assessment of the clinical usefulness of these new techniques is thought to rest on correlation with biologic behavior and responsiveness to hormone therapy.

From the 1Department of Anatomical Pathology, Hiroshima University Hospital, Hiroshima; Departments of Pathology, 2Tokai University School of Medicine, Kanagawa, 3Saitama Cancer Center, Saitama, 4Tohoku University Hospital, Miyagi, 5Dokkyo Medical University School of Medicine, Tochigi, 6Faculty of Medicine, Kagoshima University, Kagoshima, and 7Cancer Institute Ariake Hospital, Tokyo; 8Oncology and Immunology, Nagoya City University Graduate School of Medical Sciences; Nagoya; and the Departments of 9Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, 10Kyorin University School of Medicine, Tokyo, and 11Medical Oncology, National Cancer Center Hospital, Tokyo, Japan.

Supported by the task force for “Adequate evaluation for immunohistochemical evaluation in routine practice for breast cancer” established by the Japanese Breast Cancer Society, Tokyo.

Address reprint requests to Dr Arihiro: Dept of Anatomical Pathology, Hiroshima University Hospital, 1-2-3 Kasumi, Minami-ku, Hiroshima, 734-8551, Japan.

Acknowledgment: We are deeply grateful to Kyomi Nakamura for statistical calculations and drawing the figures.

References


