Immunohistochemical Measurement of Estrogen Receptors in Breast Cancer Tissue Samples

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Abstract

A new estrogen receptor immunocytochemical assay (ER-ICA) which uses monoclonal antibodies to the estrogen receptor protein was applied to 97 breast cancer tissues. The results were correlated to those obtained by conventional dextran coated charcoal assays. The presence or absence of nuclear staining was significantly associated with positive or negative estrogen receptor status by dextran-coated charcoal (P < 0.001). Furthermore ER-ICA results showed a high degree of correlation with a light microscopic grading. The relationship between ER-ICA results and response to endocrine therapies in patients with advanced disease was assessed in 20 patients. Six of 11 (55%) ER-ICA positive patients responded, whereas 8 of 9 (89%) ER-ICA negative patients failed.

Introduction

First published by Jensen et al. (1) then by Maass et al. (2), the determination of estrogen receptors in breast cancer specimens is an accepted tool for predicting response to endocrine therapies in patients with metastatic disease. Moreover, ER positive patients seem to have a longer disease-free interval and a longer survival time (3).

Conventional biochemical assays such as the DCC method are used to determine the binding capacity for estrogens or gestagens. These expensive assays require significant amounts of tissue and specific tissue handling. Usually they are performed by highly trained technicians.

The development of monoclonal antibodies to ER by Greene et al. (4, 5) helps to overcome these disadvantages. Utilizing an immunoperoxidase technique (6), King et al. (7) established a new ER-ICA that can easily be performed on frozen sections of cancer tissue specimens.

In the following report, we present our results with this newly developed assay.

Materials and Methods

Breast cancer specimens obtained from 29 women and stored at −70°C for periods ranging up to 4 years and fresh breast cancer tissue samples from 68 women were retrospectively and prospectively studied by ER-ICA. ER-ICA test kits were prepared and donated by Abbott Laboratories, Chicago, IL. A semiquantitative evaluation of immunocytochemical staining was performed using a histoscore which relates to the tumor ER content. Based on the intensity of nuclear staining a subjective integer score of 0 to 4 was given with 0 indicating no evidence of staining. To assess the average degree of staining within a tumor, various regions of the tumor section were analyzed. The second parameter was the estimated proportion of stained cancer cells in percentage. The formula for the histoscore is: histoscore = Σ (i + 1) × P, where i = intensity of nuclear staining (0–4) and P = percentage of stained cancer cells.

Specimens with a histoscore of equal to or greater than 100 were considered to be ER-ICA positive. This cutoff was based on a comparison with DCC results.

A light microscopic grading was evaluated by the method of Bloom and Richardson (8), indicating a low, medium, and high degree of aggressiveness of a given tumor. Biochemical ER was measured by DCC according to the standards of the European Organization for Research on Treatment of Cancer (9, 10).

Twenty patients with metastatic breast cancer were treated by endocrine modalities. The interval between biopsy and therapy varied from 2 to 35 months. Six patients were given tamoxifen. A total of 13 patients received high dose medroxyprogesteroneacetate. One patient was treated with tamoxifen plus medroxyprogesteroneacetate.

Clinical response to hormone therapy was determined according to the criteria of the European Organization for Research on Treatment of Cancer (11). In brief, patients were classified as complete responders, partial responders (no change), and patients with progressive disease.

Results and Discussion

The use of ER-ICA on frozen sections of ER positive breast tumors results in positive nuclear staining with little or no cytoplasmic localization of the antibody (Fig. 1). Within tumor sections heterogeneity of staining was common. In no sample were 100% of the cancer cells stained. Twenty-nine of 97 breast cancer samples analyzed were entirely negative. These observations correspond to results obtained by King and Greene (12) who demonstrated that the major portion of receptor resides in the nuclei of target cells.

According to our semiquantified ER-ICA results and our criteria for interpretation, 54 cancer samples (56%) were ER-ICA positive and 43 specimens (44%) ER-ICA negative. A comparable distribution was published by Pertschuk et al. (13).

As can be seen in Table 1, a good correlation was observed between ER status (positive or negative) obtained by ER-ICA and by conventional biochemical ER assay (DCC). We found agreement (χ² test) in ER status in 84 tumors (86%) with a P value of less than 0.001. The relationship between ER values by conventional biochemical assay (DCC) and semiquantified ER-ICA is demonstrated in Fig. 2. Although there was some agreement between low and high levels measured by DCC or ER-ICA, statistically we calculated no significant correlation. Besides other factors, this lack of significance may be caused by the small number of ER positive patients and by the crude and subjective histoscore.

The relationship between semiquantified ER-ICA (histoscore) and light microscopic grading results is shown in Fig. 3. As known from results obtained by conventional ER assays (3, 14) aggressive tumors (grade III) were significantly more often ER-ICA negative than grade I tumors.

The clinical correlation data are shown in Tables 2 and 3. Overall 7 of 20 patients (35%) with metastatic breast cancer responded objectively to endocrine manipulations. The relationship between semiquantified ER-ICA results and clinical response is shown in Table 2. Six ER-ICA positive patients...
ER-ICA ON BREAST CANCER TISSUES

Fig. 1. Nuclear immunoperoxidase staining of estrogen receptor protein in a frozen section of a tubular carcinoma.

Table 1 Comparison of estrogen receptor results in 97 breast cancer tissue samples obtained by ER-ICA or DCC assay

<table>
<thead>
<tr>
<th></th>
<th>ER-ICA positive</th>
<th>ER-ICA negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCC positive a</td>
<td>46</td>
<td>8</td>
<td>54</td>
</tr>
<tr>
<td>DCC negative</td>
<td>5</td>
<td>38</td>
<td>43</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>46</td>
<td>97</td>
</tr>
</tbody>
</table>

a ER-ICA positive: histoscore > 100 (method of semiquantification of histoscore described in text).

b DCC positive: >20 fmol/mg tissue protein.

Table 2 Response to endocrine therapy in 20 patients with advanced breast cancer correlated to semiquantified ER-ICA results

<table>
<thead>
<tr>
<th></th>
<th>Complete response + partial response</th>
<th>Progressive disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER-ICA positive a</td>
<td>6 (55) b</td>
<td>5</td>
</tr>
<tr>
<td>ER-ICA negative</td>
<td>1 (11)</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>7 (35)</td>
<td>13</td>
</tr>
</tbody>
</table>

a ER-ICA positive: histoscore > 100.
b Numbers in parentheses, percentage.

Achieved a remission whereas five ER-ICA positive patients failed therapy. In the group of ER-ICA negative patients only one responded to endocrine therapy, and eight patients failed. Regarding ER-ICA values, it seems as if patients with high levels of ER have a better chance to respond (see Table 3). Two patients which were classified ER positive by DCC assay (patients H. M. and F. H.) were ER-ICA negative and showed progressive disease under endocrine therapy. This result supports the idea that patients with biochemically receptor positive cancers who failed to respond objectively to hormone manipulations will be those whose tumors contain enough receptor negative and presumably nonhormone dependent cells to preclude an objective remission.

In conclusion the immunohistochemical determination of ER by monoclonal antibodies provides a simple, rapid, and easy to handle technique. It gives reproducible and comparable results to conventional ER assays. As reported here and by Pertschuk et al. (13) and McCarthy et al. (15), ER-ICA results can be used to predict response to endocrine therapy, even when quantification is carried out by subjective means. Before the application of ER-ICA within oncological departments can become routine, more clinical correlations of ER-ICA results and response to hormone manipulations are warranted.
### Table 3 Correlation of semiquantified ER-ICA results with clinical data and response to endocrine therapies in 20 patients with advanced breast cancer

<table>
<thead>
<tr>
<th>Patient</th>
<th>Histoscore&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ER (fmol/mg tissue protein)</th>
<th>PGR&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Biopsy site</th>
<th>Dominant site of metastasis</th>
<th>Treatment</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. H.</td>
<td>200</td>
<td>71</td>
<td>17</td>
<td>Skin</td>
<td>Soft tissue</td>
<td>TAM</td>
<td>PR</td>
</tr>
<tr>
<td>J. K.</td>
<td>360</td>
<td>132</td>
<td>0</td>
<td>Breast</td>
<td>Osseus</td>
<td>MPA</td>
<td>PR</td>
</tr>
<tr>
<td>A. M.</td>
<td>320</td>
<td>137</td>
<td>0</td>
<td>Breast</td>
<td>Osseus</td>
<td>MPA+TAM</td>
<td>CR</td>
</tr>
<tr>
<td>F. K.</td>
<td>200</td>
<td>85</td>
<td>46</td>
<td>Breast</td>
<td>Pulmo</td>
<td>TAM</td>
<td>PR</td>
</tr>
<tr>
<td>W. M.</td>
<td>160</td>
<td>122</td>
<td>28</td>
<td>Breast</td>
<td>Osseus</td>
<td>TAM</td>
<td>PR</td>
</tr>
<tr>
<td>K. B.</td>
<td>180</td>
<td>69</td>
<td>50</td>
<td>Breast</td>
<td>Osseus</td>
<td>TAM</td>
<td>PR</td>
</tr>
<tr>
<td>M. B.</td>
<td>100</td>
<td>48</td>
<td>0</td>
<td>Lymphnode</td>
<td>Soft tissue</td>
<td>MPA</td>
<td>PD</td>
</tr>
<tr>
<td>M. B.</td>
<td>120</td>
<td>76</td>
<td>10</td>
<td>Breast</td>
<td>Soft tissue</td>
<td>TAM</td>
<td>PD</td>
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<tr>
<td>W. S.</td>
<td>200</td>
<td>146</td>
<td>26</td>
<td>Lymphnode</td>
<td>Osseous</td>
<td>MPA</td>
<td>PD</td>
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<tr>
<td>B. K.</td>
<td>100</td>
<td>29</td>
<td>18</td>
<td>Breast</td>
<td>Visceral</td>
<td>MPA</td>
<td>PD</td>
</tr>
<tr>
<td>H. G.</td>
<td>100</td>
<td>78</td>
<td>0</td>
<td>Breast</td>
<td>Visceral</td>
<td>MPA</td>
<td>PD</td>
</tr>
<tr>
<td>A. H.</td>
<td>0</td>
<td>0</td>
<td>42</td>
<td>Skin</td>
<td>Soft tissue</td>
<td>TAM</td>
<td>PR</td>
</tr>
<tr>
<td>H. M.</td>
<td>20</td>
<td>60</td>
<td>15</td>
<td>Breast</td>
<td>Visceral</td>
<td>MPA</td>
<td>PD</td>
</tr>
<tr>
<td>K. B.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Lymphnode</td>
<td>Visceral</td>
<td>MPA</td>
<td>PD</td>
</tr>
<tr>
<td>F. H.</td>
<td>0</td>
<td>32</td>
<td>0</td>
<td>Skin</td>
<td>Soft tissue</td>
<td>MPA</td>
<td>PD</td>
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<tr>
<td>H. S.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Breast</td>
<td>Visceral</td>
<td>MPA</td>
<td>PD</td>
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<tr>
<td>W. S.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Skin</td>
<td>Visceral</td>
<td>MPA</td>
<td>PD</td>
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<tr>
<td>C. S.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Lymphnode</td>
<td>Soft tissue</td>
<td>MPA</td>
<td>PD</td>
</tr>
<tr>
<td>V. L.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Skin</td>
<td>Visceral</td>
<td>MPA</td>
<td>PD</td>
</tr>
<tr>
<td>H. T.</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>Skin</td>
<td>Osseous/soft tissue</td>
<td>MPA</td>
<td>PD</td>
</tr>
</tbody>
</table>

<sup>a</sup> Method of semiquantification of histoscore described in text.

<sup>b</sup> PGR, progesterone receptor; TAM, tamoxifen; MPA, high dose metroxyprogesteroneacetat; CR, complete response; PR, partial response; PD, progressive disease.

### References

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