Assessment of Results of Estrogen and Progesterone Receptor Assays Performed in a Community Hospital

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ABSTRACT

Approximately 1,000 assays for estrogen receptor (ER) in primary human breast tumors have been performed at St. Joseph’s Hospital over a period of seven years; 700 of these included assays for progesterone receptor (PR). Based on the method of analysis (dextran-coated charcoal) and criteria for a positive result used for this survey, 80 percent of the primary tumors were ER-positive and 56 percent were PR-positive. In those cases where both assays were performed, 47.4 percent were ER-positive, PR-positive; 19.8 percent were ER-positive, PR-negative; 6.2 percent were ER-negative, PR-positive; and 26.6 percent were ER-negative, PR-negative. The mean concentration of ER increased with the advancing age of the patient; essentially the same relationship was observed for PR. The concentration of ER and PR was not directly dependent upon the degree of cellularity of the tumor. Lobular carcinoma and the mixed types containing ductal and lobular elements had the highest frequency of being positive for both steroid receptors, while medullary and papillary carcinomas were lowest.

Three hundred and twenty-two cases had follow-up studies and were examined on the basis of the available information in the files of St. Joseph’s Hospital Tumor Registry. A higher survival rate in patients with both ER and PR positivity became evident.

In a community hospital setting, our data confirm the usefulness of estrogen and progesterone receptor assays in decisions of clinical management and considerations of prognosis in patients with mammary carcinoma.

Introduction

The treatment of breast cancer, the most common malignancy in white females over 40 years of age, is a subject receiving much attention, investigation, and change in opinion. Among the different surgical alternatives, modified
radical mastectomy remains the preferred treatment. Recurrences and disseminated tumors are treated by radiotherapy, cytotoxic chemotherapy, and hormonal manipulation.

For many years it was known that removal of the ovaries, adrenals or the pituitary had a beneficial effect in some cases of carcinoma of the breast. The same effect was obtained using high doses of estrogen near the toxic range. Recently, compounds almost devoid of side effects, called anti-estrogens, have become available for "hormone sensitive" tumors. Even though endocrine therapy appears to benefit only one-third of the cases, the quality of their remaining life seems to be appreciably better.

A major advantage came about when it was possible to predict with a high degree of accuracy the hormonal receptor status of each tumor. Methods for cytoplasmic estrogen and progesterone receptors were introduced, and correlative studies led to the application of the data for clinical decisions in the management of cancer patients.

The flow chart, figure 1, widely used in clinical practice for the treatment of breast cancer, indicates the importance of the laboratory reports in making therapeutic decisions. Regardless of the mass of the tumor, endocrine therapy is used first in those cases which contain hormonal receptors. In those patients whose receptor status precludes the use of hormonal treatment, chemotherapy is started immediately.

Since 1976, our laboratory has routinely performed assays for estrogen receptors in breast tumor tissue. Beginning in 1979, assays have also been performed for receptors to progesterone, since it has been shown that the predictive value is enhanced when both assays are considered together.

The analytical methods for estrogen and progesterone receptor are relatively complicated and require a great deal of handling. The quality control data of these methods may show a wide range of variability. Control tissue has only recently been made commercially available. Under these circumstances especially, the clinical utility of these assays must be closely evaluated.

The Tumor Registry has follow-up records in 322 cases of breast carcinoma diagnosed and treated at St. Joseph's Hospital (SJH) during a four year period. These records were used to derive survival statistics in relation to estrogen and progesterone receptor status.

Materials and Methods

The methods of Korenman and Duke and Johnson and Nakamura were used to assay estrogen and progesterone receptor, respectively. Tritiated estradiol or progesterone is allowed to incubate with cytosol at different concentrations of ligand. Following equilibration, unbound ligand is removed by treatment with dextran-coated charcoal. Radioactivity remaining after the removal of the dextran-coated charcoal is due to receptor bound estradiol or progesterone. Non-specific binding is that binding measurable in the presence of a saturating concentration of non-radioactive ligand and is subtracted from the total. The data are analyzed by Scatchard analysis to arrive at number and affinity of binding sites.

Sixty percent of the tests performed in our laboratory are obtained from patients...
undergoing surgery in our institution. After removal, the tissue is rushed from the operating room to the pathology laboratory where the tumor is dissected from the rest of the specimen. A representative sample is submitted for frozen section diagnosis and, when possible, one gram of tumor is diced, frozen in liquid nitrogen, and stored at —72°C. Forty percent of the samples examined consisted of already frozen specimens. Freezing was achieved in different ways, including the use of a cryostat, freon, (such as Cryokwik), and liquid nitrogen.

Based on our own experience and the generally accepted values reported in the literature, levels in excess of three femoles per mg of cytosolic protein were considered positive for estrogen receptor. Progesterone receptor values are considered positive at values greater than 10 femoles per mg of cytosolic protein.

In those cases where histologic material was available for review, the grading of each tumor was made in accordance with the guidelines suggested by Fisher, Redman and Fisher,\(^5\) taking into consideration the presence or absence of tubules in the invasive component of each tumor and the degree of cellular differentiation with grade 1 being the most differentiated and grade 3 the least. Only infiltrating ductal carcinomas were selected for correlation with receptor assay values. The tumors were also divided into three categories according to the presence of slight, moderate, or marked necrosis.

**Results**

The percent of positive hormone receptors in our cases were compared to those cases submitted to us from other hospitals. Estrogen receptor content was measured in 1,044 cases of which 540 were from St. Joseph’s Hospital (SJH) and 504 from referring hospitals (RH) (table I). Eighty percent of SJH cases and 77 percent of the RH specimens were positive. Progesterone receptors were measured in 708 cases. Fifty-eight percent of SJH and 53 percent of the RH...
Tumor Necrosis and Estrogen and Progesterone Receptor Status

<table>
<thead>
<tr>
<th>Necrosis</th>
<th>Percent ER+</th>
<th>Percent PR+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>87</td>
<td>64</td>
</tr>
<tr>
<td>Moderate</td>
<td>65</td>
<td>33</td>
</tr>
<tr>
<td>Severe</td>
<td>45</td>
<td>22</td>
</tr>
</tbody>
</table>

Histologic material was available for evaluation. Except in the very hypocellular specimen, there was no clear correlation between the cellularity of the tumor and the positivity of the sample. Grade I, well differentiated carcinoma, was more likely to have estrogen and progesterone receptors than the less differentiated, more aggressive tumors of Grades II and III (table II).

In our study, a greater percentage of mucinous, tubular, lobular, and mixed tumors with lobular elements contained greater than three femoles per mg of protein of estrogen receptors than the other types. A similar trend was noted for progesterone receptors (table III). This observation has also been made and reported in the past by several investigators.2,6,15,17

The presence of necrosis in the histologic material has consistently been associated with poor prognosis.5 Included in the same group are the two types of tumor necrosis found in breast cancer, i.e., the necrosis present in the comedo-carcinoma and the less frequent infarct-like necrosis seen in the invasive area of the tumor. Tumor positivity for estrogen and progesterone receptors was indirectly proportional to the degree of tumor necrosis (table IV).

Survival Statistics

Three hundred and twenty-two cases of carcinoma of the breast diagnosed and treated at St. Joseph's Hospital and followed by the Tumor Registry have been analyzed for survival statistics. The expected survival rates were taken from the Table of Survival Probabilities for women in the U.S. published by the National Center for Health Statistics and based on the mean patient age for each group. The observed survival rates were then expressed relative to the published Survival tables.1

Tumor estrogen receptor and progesterone receptor studies were performed

TABLE V
Evaluation of Estrogen and Progesterone Receptor Status Survival Data

<table>
<thead>
<tr>
<th>ER Status</th>
<th>Number of Cases</th>
<th>Percent of Total</th>
<th>Age Range</th>
<th>Percent of Average Age</th>
<th>Percent of Group Over 50 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER-, PR-</td>
<td>86</td>
<td>26.6</td>
<td>27-84</td>
<td>55.4</td>
<td>33.7</td>
</tr>
<tr>
<td>ER-, PR+</td>
<td>152</td>
<td>47.4</td>
<td>29-89</td>
<td>63.5</td>
<td>17.0</td>
</tr>
<tr>
<td>ER+, PR-</td>
<td>64</td>
<td>19.8</td>
<td>38-88</td>
<td>63.7</td>
<td>12.5</td>
</tr>
<tr>
<td>ER+, PR+</td>
<td>20</td>
<td>6.2</td>
<td>25-82</td>
<td>53.5</td>
<td>45.0</td>
</tr>
<tr>
<td>Total</td>
<td>322</td>
<td>100</td>
<td>25-29</td>
<td>60.8</td>
<td>22.3</td>
</tr>
</tbody>
</table>
FIGURE 3. Distribution of ER concentration in each decade.

FIGURE 4. Distribution of PR concentration in each decade.
on all patients. Appropriate therapy and follow-up were provided at SJH.

Follow-up studies of these patients ranged from one to 48 months from the time of diagnosis with 56 patients followed for four years. The actuarial method was used to calculate the survival data on a monthly basis. The cause of death was not specified. The age ranged from 25 to 89 years, all patients were female, and there was no distinction made for race. Four groups were evaluated based on estrogen and progesterone receptor status as seen on the following table (table V).

Of those patients who were both estrogen receptor and progesterone receptor negative, one third were under the age of 50 with the average age of the group being 55.4 years. The estrogen receptor and progesterone receptor positive patients had an average age of 63.5 years with only 17 percent under the age of 50 years. There is no significant difference in age distribution between the ER+PR+ and ER+PR− groups, suggesting ER positivity may be more closely related to increasing age than PR status. The group of ER−PR+ patients is too small to draw any conclusive data, although most of these patients fell into a younger group (figure 2). The concentration of ER increased with age, and those patients over 50 were more likely to have a strongly positive result (figure 3).

### Table VI

<table>
<thead>
<tr>
<th>Age Range</th>
<th>Percent ER+</th>
<th>Percent PR+</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 - 40</td>
<td>31.8</td>
<td>36.4</td>
</tr>
<tr>
<td>40 - 50</td>
<td>54.4</td>
<td>52.2</td>
</tr>
<tr>
<td>50 - 60</td>
<td>62.7</td>
<td>46.7</td>
</tr>
<tr>
<td>60 - 70</td>
<td>68.2</td>
<td>50.0</td>
</tr>
<tr>
<td>70 - 80</td>
<td>88.9</td>
<td>66.6</td>
</tr>
</tbody>
</table>

### Table VII

<table>
<thead>
<tr>
<th>ER, PR Status</th>
<th>Observed Survival Rate (Percent)</th>
<th>Expected Survival Rate (Percent)</th>
<th>Adjusted Survival Rate (Percent)</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER-, PR-</td>
<td>82.9</td>
<td>96.3</td>
<td>86.0</td>
<td>0.053</td>
</tr>
<tr>
<td>ER+, PR+</td>
<td>93.3</td>
<td>92.0</td>
<td>100.0</td>
<td>0.012</td>
</tr>
<tr>
<td>ER+, PR-</td>
<td>83.3</td>
<td>92.0</td>
<td>90.5</td>
<td>0.040</td>
</tr>
</tbody>
</table>

ER−, PR+ three year survival rate is 100 percent, the four year data are not significant. Standard error = 0.071.
Concentration of PR increased less dramatically with age (figure 4). There was a significant increase in PR positivity over 40 years of age with no further increase until 70 years of age while ER positivity increased linearly with age (table VI). The four year survival data are displayed here based on the status of estrogen and progesterone receptors (table VII).

For the group ER−PR+, only five cases were followed beyond three years, and survival statistics could not be calculated on this number.

Our data indicate that patients diagnosed and treated at St. Joseph’s Hospital with ER+PR+ status have a 93.5 percent observed four year survival rate and 100 percent ± 2.4 percent relative survival rate (at 95 percent confidence), which is above the national average for this group. Those patients who were ER−PR− or ER+PR− had an average observed survival rate of 83 percent; however, when corrected for other causes of death and age, the relative survival rates diverged. However, at the 95 percent confidence level, this difference is insignificant. The ER−PR− patients have a relative survival rate of 86.0 percent ± 10 percent and ER+PR− patients have a relative survival rate of 90.5 percent ± 8 percent. These findings suggest that PR status predicts more accurately survival prognosis than ER status (figures 5 and 6).

Further information gathered from the survival studies reveals a significant drop in survival between 12 and 30 months for all groups. This suggests a period of increased risk for recurrence in all types of carcinoma. The mortality rate observed for the entire study of 322 cases over four years was 9.31 percent. The mortality rates by group were 15.1 percent for the ER−PR− group, 4.6 percent for the ER+PR+ group, 14.1 percent for the ER+PR− group, and 5.0 percent for the ER−PR+ group. All deaths occurred prior to 30 months of follow-up.

Conclusion

The percentage of tumor epithelium present in the sample was not proportional to the levels of receptors. Our total rate of positive estrogen and progesterone receptors, as well as the proportions of the different tumor phenotypes, are in accord with most of the values published in the medical literature. As in most series, our older, post-menopausal patients had tumors that were more frequently steroid receptor positive.

Progesterone receptors have been mentioned previously as the more specific of the two markers, and our data seem to support this theory. Efforts are made to report levels of both tumor steroid receptors.

Based on our review of 322 cases of breast carcinoma at St. Joseph’s Hospital from January 1979 to December 1983, there is a significant correlation between survival and PR status. The prognostic value of the PR assay as a single test is superior to the ER assay, although ER status alone correlates more closely to age. The group ER−PR+ is small in number and thus unsuitable for analysis; however, others have suggested that this group may represent an error of the ER test itself which is not as sensitive as the PR assay. These patients, although few, did have very high survival rates and perhaps should be considered to be in the ER+PR+ category for prognostic purposes. The high survival of the ER+PR+ patients is very encouraging for the recent advances in antiestrogen therapies. Further follow-up may reveal substantial cure rates for this group.

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References