Multiple Progesterone Receptor Assays in Human Breast Cancer

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ABSTRACT

A review of assay results from more than 5500 patients revealed 283 patients in whom multiple breast cancer specimens were analyzed for progesterone receptor (PGR). All assays were performed in a single laboratory between 1975 and 1982 using the sucrose gradient technique. We considered only the 8S fraction of PGR. Simultaneous assays in 109 patients yielded 14% discordance [one assay with >10 fmol/mg cytosol protein (PGR+)+ and one assay with <5 fmol/mg protein (PGR−)]. Among 161 sequential assays, there was an overall discordance of 19%: 8% (nine of 106) when the initial assay was PGR+, but 44% (24 of 55) when the initial assay was PGR+. Among PGR+ patients initially assayed at the time of diagnosis, there was a tendency to greater receptor loss in patients with positive axillary lymph nodes (44 versus 11%). The length of time between biopsies did not increase the discordance, but endocrine therapy within this interval did increase it (56% of initially PGR+ patients who received interim endocrine therapy were PGR− at second biopsy). To evaluate the significance of interval loss of PGR, we compared survival from initial biopsy in initially PGR+ patients who subsequently lost their receptor versus those whose receptor persisted. The latter group experienced a significantly longer survival (p < 0.02). In summary, we observed an ominous loss of PGR in sequential biopsies, particularly with intervening endocrine therapy, and those patients whose tumor cells lost PGR experienced poorer survival than did patients retaining PGR. Therefore, patients with PGR+ primary tumors require repeat biopsy for PGR upon disease recurrence for optimal treatment planning.

INTRODUCTION

Hormones have been used in the therapy of breast cancer for nearly a century. Since the observations by Jensen et al. (10) in the late 1960s, that the measurement of ERs might define a group of breast cancer patients who would respond to hormonal manipulation, our ability to identify such patients has improved considerably. The response rate of ER+ patients to endocrine therapy has been repeatedly demonstrated to range between 55 and 60% (11, 16, 29). As suggested initially by Horwitz et al. (8), the addition of the PGR assay allows an improved ability to predict response to endocrine therapy, often as high as 77 to 82% in PGR+ patients (15, 16). PGR confers a reduced chance of recurrence as well, even in otherwise high-risk patients with positive axillary lymph nodes and an undifferentiated histology (23).

Because of the chronic nature of breast cancer, in which a typical case is fraught with recurrences, we can expect an increasing dependence on hormone receptor assays in treatment planning. This dependence raises new issues. From a clinician’s viewpoint, we might ask if each subsequent recurrence need be biopsied to evaluate hormone receptor status in order to optimize treatment planning. We must also improve our understanding of the impact of time, therapy, or other factors on receptor levels. The basic scientist might further question how changes in receptor status reflect true biological changes in the tumor itself.

To begin to answer these questions, various authors have studied multiple simultaneous and sequential assays for ER (1, 13, 20, 27, 30). In a recent review of 232 patients, for example, Hull et al. (9) found that major discordance between sequential biopsies was not great except in a subgroup of patients who had received tamoxifen within 2 months preceding the second biopsy, and who demonstrated a striking 78% discordance rate (first biopsy, ER+; second biopsy, ER level <3 fmol/mg protein). For PGR, however, there remains little published information about changes in multiple assays other than information on a few biopsies mentioned in the above reports (13, 20).

In the present study, we evaluate a large series of patients on whom multiple simultaneous or sequential biopsies were assayed for PGR in the same laboratory. The study reveals a striking discordance in patients whose initial biopsy was PGR+. Those patients apparently losing PGR between biopsies experienced significantly poorer survival than those retaining PGR.

MATERIALS AND METHODS

PGR Assays. During the period from 1975 to December 1982, breast cancer specimens from more than 5500 patients were evaluated for PGR content in a single laboratory. The sucrose density gradient assay was used for all specimens and, based on the work of Powell et al. (24), we focused on the 8S R5020-binding fraction as most representative of PGR. In the present analysis, a value greater than 10 fmol/mg cytosol protein was considered PGR+, a value less than 5 fmol/mg cytosol protein was considered PGR−, and intermediate PGR values were defined as having a 8S PGR level between 5 and 10 fmol/mg protein. Multiple biopsy specimens from the same patient were defined as having major discordance with respect to PGR if one specimen was PGR+ and the other was PGR−. Minor discordance was defined as one specimen PGR+ or PGR−, and the other sample having an 8S PGR level between 5 and 10 fmol/mg protein.

Patients. Multiple assays were performed on tissue from 280 patients. Multiple samples were considered simultaneous if the surgical specimens were obtained from the same patient less than 14 days apart without intervening therapy. Samples were defined as sequential if the interval between biopsies was greater than 6 weeks. The 3 patients whose biopsy intervals were between 2 and 6 weeks were excluded from further analysis. The remaining patients ranged in age from 27 to 89, with a median of 55 years; 63% were postmenopausal at the time of diagnosis. The majority presented with Stage I or Stage II disease, 32 and 56%, respectively; 12% were Stage III or IV.

Statistical Analysis. Comparisons of discordance rates and proportions were performed by the χ² test or Fisher’s exact test. Comparisons...
of the time between biopsies, quantitative PGR values, and other continuous variables were performed by the Wilcoxon rank sum or signed rank tests. Multivariate relationships between discordance of multiple PGR assays and potential predictive factors were examined using multiple logistic regression.

RESULTS

Simultaneous Assays. Multiple biopsy specimens from 109 patients fulfilled the criteria for simultaneous assays and were analyzed for PGR concordance (Table 1). Fifteen patients (14%) had a major discordance between PGR values, and 20 further patients (18%) had a minor discordance.

Discordance was further analyzed according to biopsy site (Table 2). Metastatic sites included nodes, skin, breast, liver, bone, and lung. In comparing the overall discordance rates (major and minor), we found an appreciable and comparable discordance whether samples were obtained from 2 areas of the same primary (35%) or from 2 different sites (primary versus metastasis or 2 separate metastases) (31%). This considerable variation, even within a single primary, may reflect true heterogeneity within the tumor, or a lack of reproducibility of the assay, or both.

Sequential Assays. PGR content was studied in 174 sequential pairs of assays. We found major discordance in 9% of the 106 patients whose initial biopsy was PGR− but in a striking 44% of patients whose initial biopsy was PGR+ (Table 3). Further analyses focused on the PGR+ group in an attempt to explain this high discordance rate.

We performed univariate analyses to identify factors potentially influencing PGR status, limiting our study to those patients in whom the data were obtained at the time of initial diagnosis. We first evaluated tumor size and found a substantial major discordance rate in all groups, ranging from 29% in tumors equal to or less than 2 cm in size, to 43% in tumors of 2 to 5 cm in size. These differences are not significant. There was a tendency toward greater discordance in patients with positive axillary lymph nodes; PGR+ patients with no positive axillary nodes at diagnosis had only a 11% major discordance rate, while patients with one to 3 and greater than 4 positive nodes had a greater discordance rate (56 and 39%, respectively) (p = 0.07). In evaluating the effect of menopausal status, we determined that postmenopausal patients had nearly twice the discordance rate of premenopausal patients (41 versus 23%), although the difference was not statistically significant (p = 0.28).

To evaluate the potential presence of a biological “drift” (time-dependent change) on tumor receptor status, we evaluated the impact of the time interval between biopsies (Table 4). Whether patients were initially PGR+ or PGR−, the median interval between biopsies was not significantly different for concordant versus discordant results. This suggests that a longer time interval between biopsies cannot be implicated in the evolution of tumors from PGR+ to PGR−. In fact, there is a tendency toward shorter time to second biopsy in the major discordance groups. This suggests a possible correlation between discordance and the aggressiveness (and, hence, probable rapid recurrence) of the tumor.

Intervening therapy might be expected to have a significant impact on PGR values. Our analysis of PGR− patients revealed little discordance whether or not there was intervening therapy (Table 5). Focusing on PGR+ patients, the 19 who received no intervening therapy or only chemotherapy demonstrated a major discordance of 21%. However, a startling 56% of the 27 patients who received interval endocrine therapy showed apparent loss of PGR. These findings were consistent for both adjuvant and therapeutic intervening therapy.

We were particularly interested in the impact of endocrine therapy, since tamoxifen has been associated with a remarkable apparent loss of ER in patients who had received this drug within 2 months of a second biopsy (9). In the present study, there was an 83% major discordance rate among the PGR+ patients whose endocrine therapy ended within 2 months of the second biopsy.

Table 1

<table>
<thead>
<tr>
<th>Assays</th>
<th>Rates of discordance</th>
<th>No. of patients</th>
<th>Total % of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concordance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGR+, PGR+</td>
<td>25/109 (23)</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>PGR−, PGR−</td>
<td>49/109 (45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discordance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGR−, PGR±</td>
<td>Minor</td>
<td>9/109 (8)</td>
<td>18</td>
</tr>
<tr>
<td>PGR±, PGR+</td>
<td>11/109 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGR−, PGR+</td>
<td>Major</td>
<td>15/109 (14)</td>
<td>14</td>
</tr>
</tbody>
</table>

Numbers in parentheses, percentage of patients with a given assay type.

Table 2

<table>
<thead>
<tr>
<th>Simultaneous assays and discordance by biopsy source</th>
<th>No. of patients with minor discordance</th>
<th>No. of patients with major discordance</th>
<th>Total no. of patients with discordance</th>
<th>Total no. of patients</th>
<th>Total % of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary, primary</td>
<td>10/51 (20)</td>
<td>8/51 (16)</td>
<td>18/51 (35)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Primary, metastasis</td>
<td>3/22 (14)</td>
<td>2/22 (9)</td>
<td>5/22 (23)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Metastasis, metastasis</td>
<td>3/10 (30)</td>
<td>2/10 (20)</td>
<td>5/10 (50)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Unknown site</td>
<td>4/26 (15)</td>
<td>3/26 (12)</td>
<td>7/26 (27)</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Numbers in parentheses, percentage of patients with a given assay type.

Table 3

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Sequential assays</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGR+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concordance</td>
<td>27/55 (49)</td>
<td></td>
</tr>
<tr>
<td>Minor discordance</td>
<td>4/55 (7)</td>
<td></td>
</tr>
<tr>
<td>Major discordance</td>
<td>24/55 (44)</td>
<td></td>
</tr>
<tr>
<td>PGR−</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concordance</td>
<td>94/106 (89)</td>
<td></td>
</tr>
<tr>
<td>Minor discordance</td>
<td>3/106 (3)</td>
<td></td>
</tr>
<tr>
<td>Major discordance</td>
<td>9/106 (8)</td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage of patients with a given assay type.

Table 4

<table>
<thead>
<tr>
<th>Initial assay</th>
<th>Concordance</th>
<th>Minor discordance</th>
<th>Major discordance</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGR+</td>
<td>746 days (27)</td>
<td>1070 days (4)</td>
<td>550 days (24)</td>
<td>0.51b</td>
</tr>
<tr>
<td>PGR−</td>
<td>445 days (94)</td>
<td>375 days (9)</td>
<td>227 days (3)</td>
<td>0.62</td>
</tr>
<tr>
<td>Total</td>
<td>512 days (121)</td>
<td>589 days (13)</td>
<td>520 days (27)</td>
<td>0.71</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number of patients demonstrating a given assay response.

b Reflects comparison between concordance and major discordance groups.
Table 5
Sequential assays: PGR changes by intervening therapy

<table>
<thead>
<tr>
<th>Initial biopsy</th>
<th>Interval therapy</th>
<th>Minor discordance</th>
<th>Major discordance</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGR-</td>
<td>None</td>
<td>0/31 (0)</td>
<td>4/31 (13)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Chemotherapy</td>
<td>2/28 (7)</td>
<td>2/28 (7)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Endocrine</td>
<td>0/10 (0)</td>
<td>0/10 (0)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Both&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1/24 (4)</td>
<td>0/24 (0)</td>
<td>0</td>
</tr>
<tr>
<td>PGR+</td>
<td>None</td>
<td>2/16 (13)</td>
<td>4/16 (25)</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Chemotherapy</td>
<td>0/3 (0)</td>
<td>0/3 (0)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Endocrine</td>
<td>1/13 (8)</td>
<td>6/13 (62)</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Both&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1/14 (7)</td>
<td>7/14 (50)</td>
<td>40</td>
</tr>
</tbody>
</table>

<sup>a</sup> Numbers in parentheses, percentage of patients with a given assay type.
<sup>b</sup> Both endocrine therapy and chemotherapy.

However, we also found a 40% major discordance rate in the patients whose treatment with endocrine therapy had been stopped longer than 2 months prior to the second biopsy. Since the majority of these patients (20 of 27) had received tamoxifen, we next analyzed only those with intervening tamoxifen therapy (Table 6). The same pattern was observed: 75% major discordance if tamoxifen therapy within 2 months of biopsy but also 43% discordance if discontinued for at least 2 months. Fourteen patients who were initially PGR— also received tamoxifen, and all remained PGR— at second biopsy. Thus, intervening therapy apparently did not induce synthesis of PGR, although a PGR increase had been demonstrated by Namer et al. (21) with short-term use of tamoxifen. The data suggest a sensitivity of PGR to endocrine therapy which is broader and less time specific than that reported for ER. In fact, a multivariate analysis of the possible factors contributing to discordance in PGR+ patients (menopausal status, nodal status, tumor size, and intervening therapy) demonstrated intervening endocrine therapy to be the only variable of significance.

In comparing initially hormone receptor-positive patients, the 44% discordance rate in our study of sequential PGR assays was significantly higher than the 19% discordance rate reported in a large series of sequential ER assays (9). In an attempt to determine if the PGR assay identified a subset of ER patients whose assay results would be as labile as the PGR values, we analyzed the ER discordance of the patients in our study group as a function of their PGR values (Table 7). We excluded patients who had received tamoxifen within 2 months of the second biopsy because of the consistent apparent loss of ER demonstrated in this treatment group (9). If the initial PGR was positive and remained so at second biopsy, a positive ER on the same specimen remained positive in 91% of the cases. If an initial PGR+ assay was discordant, then there was an ER discordance of 47%. It appears that the PGR assay selects a subset of ER patients from the time of first biopsy in 3 groups: (a) those initially PGR— who remained so; (b) those initial PGR+ who remained so; and (c) those initially PGR+ who experienced a loss of PGR as determined by sequential biopsies. As expected, the poorest survival was seen in the group of patients who were consistently PGR—. Of greatest interest, however, we found that patients who were initially PGR+, but who lost their receptor between biopsies, had a significantly poorer survival than did those who remained PGR+ (<p> = 0.02) (Chart 1). The estimated median survival times, based on actuarial survival analyses, were 36, 76, and 51 months, respectively, in the 3 groups.

**DISCUSSION**

**Simultaneous Assays.** Discordance for simultaneous PGR assays may result from inadequate sample size, lack of assay reproducibility, or tumor heterogeneity.
Van Netten et al. (28) have pointed out that an accurate measurement of ER depends on having an adequate amount of tumor to assay. The importance of obtaining a sufficiently large tumor sample was confirmed by Hull et al. (9), who described a 56% major discordance rate in patients with an ER level of <3 fmol/mg protein, whose initial tumor was <2 cm in size, in comparison to a 7% discordance rate in tumors >2 cm, suggesting a “false-negative” ER assay in the smaller tumors. It seems evident that an adequate tumor sample would also be necessary to perform an accurate assay for PGR.

The sucrose density gradient technique is an accepted standard, although lengthy, assay for PGR (22, 24). Variability inherent in the assay technique itself nevertheless remains a possible explanation for at least a part of the observed discordance.

Finally, heterogeneity within the tumor may also contribute to discordant assay results. Investigators (3, 12) have suggested that, in any breast cancer, 2 cell populations are present: one responsive and the other unresponsive to hormone therapy. Since the presence of intracellular hormone receptor reflects tumor responsiveness to endocrine therapy, we would expect a tumor composed of cells of mixed responsiveness to have a population of cells differing in receptor content as well. Thus, our finding of the same PGR discordance from simultaneous samples within a single primary lesion and between distinct lesions might reflect, in part, the essential heterogeneity of breast cancer.

Our major discordance rate of 14% of simultaneous PGR assays is considerably higher than the 3% reported for ER (9). However, this serves as a useful base line for evaluation of PGR discordance between sequential biopsies.

Sequential Assays. The major discordance rates for sequential assays were 9% when the first specimen was PGR− but a striking 44% when the first sample was PGR+.

What is the explanation for the high discordance rate in PGR+ patients? We considered the impact of “biological drift,” or gradual loss of PGR over time; however, the lack of correlation between discordance rates and the interval between biopsies suggest that factors other than simple biological drift may play a role. An interval change in endogenous hormone balance or an effect of intervening therapy might explain the loss of PGR.

The effect of the endogenous hormonal milieu on receptor status has been discussed by Bloom et al. (2), who suggested that some postmenopausal patients, because of low circulating endogenous estrogens, may have dormant and, hence, unmeasurable PGR. By treating a group of apparently ER+, PGR− patients with exogenous estrogen, they were indeed able to stimulate the synthesis of PGR. The analysis by Clark et al. (4) of factors affecting PGR does suggest a decrease of PGR in early menopause; however, given the short interval between biopsies in our series, too few patients could have changed menopausal status to impact on our results. It has also been shown that, in premenopausal patients, fluctuations in endogenous progesterone levels may influence PGR values. Saez et al. (25) noted that PGR was apparently absent in all patients whose plasma progesterone was greater than 100 ng/ml. Although we do not have access to progesterone levels in our patients, the group of women who were premenopausal at the time of diagnosis displayed the lowest discordance rate, making such an explanation less likely.

Our analyses demonstrate clearly that intervening endocrine therapy is the single most important factor influencing the PGR discordance rate. We have described a major discordance rate of 56% in initially PGR+ patients who received endocrine therapy prior to second biopsy. Furthermore, a multivariate analysis of various factors with potential impact on PGR status shows that intervening endocrine therapy alone is the only variable which independently predicts discordance rate. Unlike the ER data of Hull et al. (9), which demonstrated a 78% discordance rate in ER+ patients receiving tamoxifen within 2 months of second biopsy but no effect of other hormonal therapy, our results for PGR show a more general effect of endocrine therapy. Although we did find a 75% PGR discordance in patients receiving tamoxifen within 2 months of biopsy, there was also a 42% discordance rate in patients whose tamoxifen therapy had ended at least 2 months prior to second biopsy, and a 57% discordance among PGR+ patients who received endocrine therapy other than tamoxifen.

The duration of tamoxifen therapy seems to determine its impact on PGR. Namer et al. (21) studied the effect of 1 week of tamoxifen on PGR status from biopsies of cutaneous metastatic lesions. They demonstrated an increase in PGR at second biopsy in the patients who had been ER+ (and, thus, presumably had hormone-responsive tumors) at first biopsy. These data suggest that short-term tamoxifen has an estrogenic effect in stimulating synthesis of PGR. Similar results have been obtained using either MCF-7 human breast cancer cells in tissue culture or isolated uteri of ovariectomized rats, in which a brief exposure to tamoxifen increased PGR levels (5, 7). By contrast, in our study, the loss of PGR after long-term treatment of patients with tamoxifen suggests that its initially positive estrogenic effect is eventually superseded by inhibition of PGR synthesis.

The key issue, however, is whether intervening endocrine therapy induces an “artificial” lowering of hormone receptor as described for ER by Hull et al. (9) or an actual change in the biological behavior of breast cancer. The presence of ER in breast cancer cells has been correlated with slower proliferation (17, 18, 26) and with several morphological features of well-differentiated tumors, including the absence of necrosis or lymphocytic infiltrates and the presence of elastica within the tumor (6). Mills (19) and McCarty et al. (14) have reached similar conclusions with regard to PGR. In fact, McCarty et al. (14) state that the nuclear grade of the tumor has an excellent correlation with the PGR level. Based on these findings, it would appear that a tumor which loses its PGR is, in fact, experiencing an ominous evolution to a more virulent cancer. Our retrospective survival analysis supports this conclusion. A PGR+ patient who loses receptor between sequential biopsies has a prognosis intermediate between those whose biopsies are consistently PGR+ or PGR−. This strongly supports the conclusion that loss of PGR reflects a change in the biological behavior of the tumor.

In this regard, the concept of a bimodal population of cells within a given tumor is relevant. It would appear that, in many cases, endocrine therapy selectively eliminates hormone-sensitive cells from a tumor, leaving a residual aggressive hormone-insensitive cell population. Whether cellular dedifferentiation and active proliferation are accelerated by the elimination of the hormone-responsive subpopulation of cells is a testable hypothesis.

Without minimizing our results, it is necessary to confront an important limitation to this type of study. There are unavoidable biases in our selection of patients. The vast majority of patients with breast cancer are rendered apparently free of disease with their initial surgical treatment. Not all will relapse. By design, our
study eliminates those cured by their initial therapy, whose tumors must be qualitatively different from those which relapse. Similarly, at the time of relapse, we are limited to performing assays only on tissue from those sites which are easily accessible to biopsy without subjecting an ill patient to undue morbidity. We would, therefore, expect to assay considerably more material from skin, soft tissue, and lymph nodes than from radiographically suspected, but surgically inaccessible, metastases in bone and brain. In a large series, Sledge et al.4 have found a tendency toward hormone receptor positivity in exactly those tissues which are most accessible to biopsy. This suggests that our sample may be biased toward PGR+ metastases, making the high discordance rate of initially PGR+ samples even more striking.

In summary, we have observed an ominous loss of PGR in sequential breast cancer biopsies from many patients, suggesting profound changes in the biological behavior or aggressiveness of their tumors, especially after intervening endocrine therapy. Indeed, those patients whose tumor cells lost PGR experienced significantly poorer survival than do patients retaining PGR. Therefore, patients with PGR+ tumors require repeat biopsy for PGR upon disease recurrence for optimal treatment planning.

ACKNOWLEDGMENTS

We wish to thank Judy Wenzel and Sylvia E. Jasso for their tireless efforts in data collection and management, Darlene Spencer for her assistance with computer programming, and Pam Render for her patience in preparing this manuscript.

REFERENCES


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