Endocrine Therapy for Advanced Carcinoma of the Breast: Effect of Tumor Heterogeneity and Site of Biopsy on the Predictive Value of Progesterone Receptor Estimations

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ABSTRACT

This study was carried out to assess the influence of site of biopsy and tumor heterogeneity upon the value of progesterone receptor (PR) measurements for prediction of response of patients with advanced carcinoma of the breast to tamoxifen or ovarian ablation.

One hundred eighty-one assessable patients were studied. Sixty-nine % of responders and 31% of nonresponders were PR positive. The times to progression and survival were not significantly different for responders whether they were receptor positive or receptor negative. PR was measured on operable primary tumors (97), inoperable primary tumors (59), and secondary deposits (69). The proportion of responders who had PR-positive biopsies from these sites was 59%, 88%, and 61%, respectively, and the proportion of PR-positive nonresponders was 30%, 27%, and 42%, respectively. Ten to 12 separate PR measurements were made on seven tumors, and in four of them, there were PR-positive and PR-negative areas which could account for false positive and false negative results.

We conclude that the optimum prediction of response was seen when the biopsy was performed on inoperable primary tumors. Errors in prediction of response at this site were, in part, explained by within-tumor heterogeneity of PR; the greater errors in prediction when measurements were made on operable primary tumors or on secondary deposits are presumed to be related to the additional effects of changing receptor status with time and between-tumor-site heterogeneity, respectively.

INTRODUCTION

Assays of PR have been widely recommended as a means of predicting endocrine response in patients with mammary cancer. Synthesis of PR is controlled by estrogens, and its presence suggests that the mechanism of estrogen response is intact (1). In about 75% of patients, assays of PR predict the effect of endocrine therapy correctly, but in the remainder the test gives misleading results (2). Such errors of prediction could arise in several ways: (a) errors of measurement; (b) heterogeneity between sites and within tissue; (c) insufficient estrogenic stimulus for synthesis of PR; and (d) autonomous synthesis of PR.

Errors of measurement should be minimized within an experienced laboratory. The extent of heterogeneity, on the other hand, is beyond control. Estimates of variation of PR between sites and within tissue; (c) insufficient estrogenic stimulus for synthesis of PR; and (d) autonomous synthesis of PR.

PR would be greatest when carried out on an undisseminated tumor shortly before endocrine treatment. Similarly the predictive value would fall when classification of PR was carried out early in the course of the disease, at a long interval before endocrine treatment became necessary, or when carried out after dissemination on only one of perhaps many secondary deposits.

In this study we have set out to determine whether the predictive value of assays of PR varied according to the source of sample used for classification, and to investigate whether the degree of tumor heterogeneity was sufficient to explain such misclassifications.

PATIENTS AND METHODS

Patients. Between November 1975 and December 1983, 670 patients with advanced carcinoma of the breast were treated. Treatment was excision and radiotherapy for local recurrence (61 of these have had no further relapse), chemotherapy for aggressive disease (n = 94), and endocrine therapy for the remainder (n = 429). Endocrine therapy was either ovarian ablation or tamoxifen in all but 36 of the cases.

Patients were selected for this study if they (a) had had progesterone receptor measurements performed upon the primary tumor or a secondary deposit; (b) were treated with tamoxifen or ovarian ablation as first systemic treatment for advanced disease; and (c) were evaluable for response according to criteria of the International Union against Cancer. Patients with stable disease for more than 6 mo were regarded as responders (12). One hundred eighty-one patients fulfilled these criteria and were derived from a group of 393 patients treated with tamoxifen or by ovarian ablation. Of those excluded, 115 were not evaluable for response, 82 were evaluable but had not had PR measured, and 15 had had adjuvant tamoxifen or ovarian ablation. There was no significant difference in the time to progression or survival between evaluable and nonevaluable endocrine-treated patients or between those with or without PR measurements. Thus the selected group are representative of the whole group of patients with advanced disease treated by tamoxifen or ovarian ablation. Thirty-four of 181 patients had adjuvant chemotherapy: all were postmenopausal.

Receptor Analysis. Biopsy material was placed immediately in liquid nitrogen. Most analyses were performed using the DCC technique and Scatchard analyses (n = 160) (13). Some analyses (n = 21) were performed using IEF when the sample was too small for a DCC assay (14).

Results of all assays were expressed as fmol/mg of cytosol protein. For both ER and PR the sample was regarded as positive if 5 or more fmol/mg of cytosol protein were measured using the DCC assay, and any positive value of appropriate pi, using the IEF assay.

Intratumor Variation of PR. Seven primary tumors in which malignancy had been confirmed by needle biopsy before operation were dissected from the breast immediately after mastectomy, and 2- to 4-mm slices were taken across the equator. The slice was trimmed of nontumor tissue and frozen in liquid nitrogen. Most analyses were performed using the DCC technique and Scatchard analyses (n = 160) (13). Some analyses (n = 21) were performed using IEF when the sample was too small for a DCC assay (14).

Results of all assays were expressed as fmol/mg of cytosol protein. For both ER and PR the sample was regarded as positive if 5 or more fmol/mg of cytosol protein were measured using the DCC assay, and any positive value of appropriate pi, using the IEF assay.

Statistical Analysis. The log rank test (15) was used to compare survival and the probability of progression for receptor subgroups.
RESULTS

The response categories for all patients and according to treatment by either tamoxifen or ovarian ablation are shown in Table 1. Twenty-nine percent of patients had a complete or partial remission, and the disease of a further 22% was stable for at least 6 mo. The time to progression and survival of patients with stable disease were not significantly different from those with partial remissions (Fig. 1), and the proportion of patients with estrogen and progesterone receptor-positive tumors and their median values were similar in these two categories and quite different from patients with progressive disease (Table 2). In addition their ER and PR receptor phenotypes were similar (Table 3).

Sixty-nine percent (64 of 93) of patients who responded to either tamoxifen or ovarian ablation and 31% (27 of 88) of those who did not respond had PR-positive tumors (Table 2). Patients were divided into four categories: PR-positive responders; PR-negative responders; PR-positive nonresponders; and PR-negative nonresponders. Fig. 2 shows that responsiveness is more important than receptor status because the times to progression and survival were similar for responders irrespective of receptor status and for nonresponders, irrespective of receptor status.

Site and Timing of Biopsy. The proportions of progesterone receptor-positive tumors for each site (operable primary, inoperable primary, and secondary deposit) and for each response category are outlined in Table 4. PR was most predictive when measured on inoperable primary tumors: 88% of responders and 27% of nonresponders had PR-positive tumors. The equivalent figures for operable primary tumors were 60% and 30% and for secondary deposits were 61% and 42%.

Tumor Heterogeneity. The results of PR measured by the DCC and IEF methods on 10 to 12 areas from 7 tumors are shown in Table 5. The IEF method gave consistently lower results than the DCC method, but the proportion of samples which were receptor positive was similar (57 of 81 for DCC and 58 of 81 for IEF). Using the IEF method three tumors were uniformly PR positive, and four had receptor-positive and receptor-negative areas. Using the DCC method, four tumors were uniformly PR positive, one uniformly PR negative, and two had receptor-positive and -negative areas. If each value was
paired with every other one within the same tumor and the results from all tumors were pooled, 12% (DCC) and 14% (IEF) of the pairs were discordant in that one was PR positive and the other PR negative.

If we assume that the three tumors with the lowest receptor values were endocrine unresponsive (H. B., M. G., and L. La.), 9 of 33 (27%) of receptor estimations on these three tumors were “falsely” PR positive when measured by the DCC method, and 12 of 33 (36%) were falsely PR positive when measured by the IEF method.

**DISCUSSION**

All patients in this study had had no previous systemic treatment for advanced disease and were treated with tamoxifen or ovarian ablation, only, in order to eliminate possible variability introduced by previous treatment and treatment with different types of endocrine therapy. Inclusion of stable disease in the response category is unusual but appears justified since patients in this category had similar receptor status, duration of remission, and survival as those with objective partial remission; other groups have reported that stable disease patients in this category had similar receptor status, duration of response and survival from the start of endocrine therapy. Inclusion of stable disease patients reviewed by McGuire and Clark (2) treated with aminoglutethimide (16) and megestrol acetate (17).

In this study 69% of responders and 31% of nonresponders who have had no intervening therapy are pooled from the primary and soft tissue metastases or between soft tissue metastases biopsied synchronously has been reported to range between 13 and 35% with a mean of 22% discordance (65 of 293); see Refs. 3-10. When PR was measured on metastases in previous studies (6, 7, 9–11) where PR was measured on metastases biopsied synchronously it has been reported to range from 25 to 44% (36 of 60).

In summary, the prediction of response by PR measurements on inoperable primary tumors is good, and within-tumor heterogeneity may account for some lack of precision; however, the prediction is relatively imprecise when PR is measured on operable primary tumors and on secondary deposits; this may be related, in part, to within- and between-tumor heterogeneity and change in receptor status with time.

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**REFERENCES**

ENDOCRINE RESPONSE AND PROGESTERONE RECEPTOR HETEROGENEITY

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