A Proposed Model for the Prediction of Response to Endocrine Therapy in Breast Cancer from the Estrogen Receptor Status of One Site and the Number of Metastatic Sites

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ABSTRACT

A mathematical model for prediction of response to endocrine therapy of breast cancer has been developed based on a clonal concept of metastatic spread. The model includes an expression of the likelihood of response of an estrogen receptor-positive site and an expression of the concordance of receptor assays when multiple sites are assayed. Both of these are raised to a power function based on the number of sites of metastases to yield a predicted response rate. An excellent fit of the predictions of this mathematical model and response data from a series of patients receiving endocrine therapy was observed. This model provides a worthwhile insight into the biology of response to endocrine therapy. The model may be extended and refined through additional analyses.

INTRODUCTION

The ER status of a tumor in a patient with metastatic breast cancer is now accepted as a useful biochemical marker for selecting patients for endocrine therapy. The response rate for hormone therapy in metastatic breast cancer overall is about 30%. However, patients whose tumor is ER+ have a response rate of approximately 60 to 65% and in patients whose tumor is ER−, the response rate is less than 10% (11).

Many factors have been discussed to explain why the response rate in ER+ tumors is not 100%. (a) The binding of hormone to receptor is only the first step in steroid hormone action. For the hormone to cause phenotypic changes in its target tissue, it must not only have an accessible receptor, but also the hormone-receptor complex must be capable of nuclear translocation and specific genomic activation. The presence of cytoplasmic ER is therefore a necessary but not a sufficient criterion for hormonal response. A tumor which contains cytoplasmic ER but which has a translocation defect would not be a hormone-dependent tumor. (b) Another consideration is tumor heterogeneity. A sufficient number of tumor cells in a sample may contain ER activity to give a positive assay result, but they may represent a minority of the total tumor cell population. Recently, several groups have shown by direct histochemical methods that tumors are generally a mixture of ER+ and ER− cells (12).

(c) It is possible that some endocrine therapies may act via some other receptor mechanism. The presence of this other receptor mechanism may correlate generally with the presence of the estrogen receptor, but the correlation between response and the ER may be less than perfect. (d) The presence of receptor may truly connote hormone responsiveness, but the therapy itself may inadequately alter ambient hormone concentrations for a response to occur. An example is the observation of a 10 to 15% response to adrenalectomy in patients who had not responded to oophorectomy. (e) Assay error is a possibility.

(f) Another possibility is that the ER status of the site which was biopsied for the ER assay does not represent the ER status of other sites of disease. We postulate that clones of tumor with differing receptor status could exist in a patient and could explain the less than 100% response rate. The hypothetical background for this postulation is that there is genetic instability within a tumor population, permitting the evolution of subpopulations which differ as to the biological characteristic under study (13), which in the present case is the ER status. Additional theoretical assumptions are that the ER status does not provide a selective survival advantage, so that subpopulations survive irrespective of their ER status, and that metastases may develop from either an ER+ or an ER− subpopulation.

This paper will present a mathematical model based on this clonal hypothesis. Using concordance data from simultaneous ER assays from multiple metastatic sites, the response rate as a function of number of metastatic sites was tested for goodness of fit with the proposed mathematical model.

The Mathematical Model

The basic assumptions of the model are:

1. If the ER is present in a given metastatic site, the chance of a response of that site to hormone therapy is close to 100%, and if the receptor is absent, the response of that site is zero. The likelihood of response of an ER+ site (labeled Q) will be estimated as part of our mathematical model.

2. A patient will be called a responder to hormone therapy only if all indicator sites contain the ER since any involved site which lacks the ER would be expected to continue to grow following introduction of the hormone therapy, and the patient would then be called a nonresponder (the definition of response requires that no areas progress). A possible exception to this assumption is the situation in which an ER− site has a very slow growth rate and appears to remain stable while other sites regress (such a patient could be called a responder). The requirement of response extending over 2 months (see below) makes this exception a very unlikely possibility.

3. The ER status for multiple lesions of the same patient will tend to be either concordantly positive or concordantly negative, and nonconcordance may result from independent reali-
zations of a probabilistic process. Patients for whom sites tend to be ER+ will be referred to as "intrinsically" ER+. For every intrinsically ER+ patient, each specific lesion is ER+ with probability $P$ (close to 1) independent of the status of the other lesions. Similarly, each specific lesion of all intrinsically ER− patients is ER− with the same probability $P$ independent of the status of the other lesions. The intrinsic ER status is merely a term for distinguishing between patients whose lesions tend to be ER+ and those whose lesions tend to be ER−. It is also a construct for defining a binomial model of concordance, but it does not imply any specific biological mechanism. Generally, the ER status of the predominant clone of the primary tumor could be taken as determining the intrinsic status of the patient.

4. Each indicator site is either homogeneous with regard to ER status of its tumor cells, or there is a predominant clone within the site whose response determines the response of the site.

5. The final assumptions have to do with counting of number of sites. Lymph node areas which could have received independent lymph drainage (clones) from the breast were counted as separate sites. However, for distant organs, each organ was counted as one site. This is in part based on the occasional difficulty in distinguishing the number of foci of involvement in an organ but more importantly on the demonstrations by Fidler (5) of the biological similarity of metastases to a given target organ.

From these assumptions, we formulate a mathematical model as follows. If the primary site has been assayed and if its ER status is taken as defining the intrinsic ER status of the patient, then the following results apply. If the resected primary site is ER+, the probability that all other $n$ sites are positive is $P^n$. Consequently, the probability that the patient will respond to hormonal therapy is

$$P^nQ^n \quad (A)$$

The $Q^n$ factor is the probability that ER+ sites respond (based on Assumption 1 above).

If the resected primary site is ER−, then the probability that the patient will respond is

$$(1 - P)^nQ^n \quad (B)$$

The first factor $(1 - P)^n$ is the probability that all $n$ indicator sites are nonconcordant with the primary site, that is, are ER+.

For most of our data, the index lesion biopsied was not that of the primary site. Consequently, application of the above expressions becomes more complex. If the biopsied metastasis is ER+, it is possible that the primary site was ER+ or ER−. Thus, the probability of response is a weighted average of Expressions A and B. The weighting factor for Expression A is

$$w_A = \frac{\Pi P}{\Pi P + (1 - \Pi)(1 - P)} \quad (C)$$

where $\Pi$ denotes the proportion of the population of patients that are expected to have ER+ primaries. This weighting factor represents the probability that the primary site is ER+, given that the index lesion was found to be ER+. The numerator of Expression C is the probability that a patient will have both an ER+ primary site and index lesion. The second term in the denominator is the probability that a patient will have an ER− primary site but an ER+ index lesion. Consequently, if the metastatic index lesion is ER+, the probability that the patient will respond equals the weighted average of Expressions A and B with the weighting factor for Expression A being Expression C, and the weighting factor for Expression B being 1 minus Expression C, or

$$w_A P^nQ^n + (1 - w_A)(1 - P)^nQ^n \quad (D)$$

It is assumed that the biopsied metastasis is not one of the $n$ sites evaluated for response. If the metastatic index lesion is ER−, there is a possibility that the primary tumor was ER+. The probability of response is again a weighted average of Expressions A and B.

Analogous reasoning to that given above shows that the weight for Expression A is

$$w_A = \frac{\Pi(1 - P)}{\Pi(1 - P) + (1 - \Pi)P}$$

Consequently, the probability that the patient will respond equals

$$w_A P^nQ^n + (1 - w_A)X(1 - P)^nQ^n \quad (E)$$

If an estimate $\Pi = 0.5$ is used, Expressions D and E simplify to approximately

$$P^n + Q^n \quad (D')$$

and

$$[(1 - P)P^n + P(1 - P)^n]Q^n \quad (E')$$

If data are available on $N$ patients, each of whom had ER determinations on 2 lesions, then the parameter $P$ can be estimated via the concordance rate. If one of the biopsied lesions for each patient is the primary site, then we estimate

$$\hat{P} = N_c/N$$

where $N_c$ denotes the number of concordant patients. This estimate is just the observed concordance rate. For our concordance data, the primary site was rarely one of the biopsied lesions, and hence this simple relation does not apply. For concordance data on metastatic lesions, the parameter $P$ can be estimated as

$$\hat{P} = 0.5 + \sqrt{\frac{N_c - 0.5}{N}}$$

This can be seen as follows. The probability of a concordant + + pair is $\Pi P^2 + (1 - \Pi)(1 - P)^2$, whereas the probability of a concordant − − pair is $\Pi(1 - P)^2 + (1 - \Pi)P^2$. The sum of these 2 expressions is the probability of a concordant pair of either type and equals $P^2 + (1 - P)^2$. Equation F results from setting this sum equal to the observed concordance rate

$$P^2 + (1 - P)^2 = N_c/N$$

and solving for $P$. This gives the maximum likelihood estimator of $P$ when the data are reported only in terms of numbers of concordant pairs. The approximate standard error of $\hat{P}$ is

$$1/[2N(2\hat{P} - 1) \sqrt{N_c + 1/N_c}]$$

where $N_c$ denotes the number of discordant patients (B).
ER Assays

The ER assay performed in our laboratory has been previously described in detail (2). Briefly, the dextran-coated charcoal technique is used under nonexchange conditions. Any tumor containing greater than 10 fmol/mg cytoplasmic protein is considered ER+, and any tumor containing less than 10 fmol/mg cytoplasmic protein is considered ER−. The validity of the assay technique and the prospectively chosen cutoff value for ER positively was ascertained in a clinical trial in which 65% of ER+ patients responded to endocrine therapy, while only a 9% response rate was seen with an ER− tumors (3). The assay technique has also been validated using the NSABP test powders (Provided by Dr. J. Wittliff, University of Louisville, Louisville, Ky.). The assay as performed in our laboratory is extremely precise. Multiple assays on MCF-7 cells grown in charcoal-treated calf serum yielded a mean ± S.E. for unoccupied cytosol receptor of 219 ± 17 fmol/mg cytoplasmic protein.

Response Criteria

In all cases, assessment of response was performed using standardized response criteria (4). In brief, complete response required the disappearance of all measurable disease, including healing of all bone lesions and a return of the patient to a premorbid performance status. Partial response required a shrinkage of at least 50% in all measurable disease. Although a given lesion might not regress to this extent, regression averaged over all lesions had to be equal to or greater than 50%. No new lesions could appear and no growth could be observed in a preexistent lesion. For purposes of this study, no patient was classified as a partial responder unless improvement was maintained for 2 months or more. Only patients with complete or partial responses are termed responders. Any patient not achieving this degree of improvement (at least 50% tumor shrinkage) was termed a nonresponder. These criteria differ from those of the International Union against Cancer (6) which define a "no change" group. In our criteria, stable disease would be defined as a failure. All patient records were examined and assessed by individuals unaware of ER results.

Results Testing the Model

The data on concordance are drawn from a recent publication (1), which is in agreement with data from other laboratories (Table 1). When 2 sites were biopsied concurrently, there was concordance of results in 23 of 27 patients (85%). When the biopsies were asynchronous, the concordance rate was 4 of 5 if there had been no interval therapy and 16 of 19 (84%) if the patient had received interval chemotherapy. Combining these results gives an overall concordance rate of 0.84. The resulting estimate of $P$ from Expression F is 0.914, and the approximate S.E. is 0.031. Thus, approximate 95% confidence limits for $P$ are from 0.852 to 0.976.

The patient response data are shown in Table 2 (3). Based on $P$ estimated from the concordance data and upon Expressions D' and E', the value of $Q$ which maximizes the likelihood function for patient response data was determined to be 0.933. The likelihood function is the probability of obtaining the response rate data shown in Table 2, conditional upon the number of patients studied in each ER status and number of indicator sites category, and expressed as a function of the unknown parameter $Q$. $Q$ is the estimated probability of response for an ER+ site (see Assumption 1 above). Predicted response rates are shown in Table 3. The approximate standard error of the estimate of $Q$ is 0.043 (8). Thus, approximate 95% confidence limits for this parameter are from 0.847 to 1.0.

The agreement between predicted and observed response rates for patients with only one involved site is quite striking both for the ER+ and ER− patients. Within the ER+ series, the response rate declines with increasing number of involved sites ($p < 0.05$ by Kruskal-Wallis test) (9), as predicted by the model. However, for ER+ patients with more than 3 sites, there was no further decline in response rate, so the data for 3 or more sites are combined in Table 2. The observed response rate for this pooled group falls between the response rate predicted for 3 and 4 sites of involvement. Within the ER− series, the response also declines with more sites involved, as predicted by the model. Differences between observed and predicted responses are in part due to the small number of responders (one for each site group). It is of interest that the one response observed in the ER− group with 3 or more sites was in a patient with 3 sites of involvement.

The goodness of fit of the observed and predicted response

![Table 1](attachment:image1.png)

![Table 2](attachment:image2.png)

![Table 3](attachment:image3.png)
rate data was evaluated using the Pearson goodness-of-fit test (14). A $\chi^2$ of 1.34 with 5 degrees of freedom was obtained. (This statistic takes into account that the parameter $Q$ was estimated from the data.) This result corresponds to a probability of greater than 0.90 that random deviations between observed and predicted responses at least as great as those obtained would be found if the model is correct. The Pearson goodness-of-fit test contains a large sample approximation, so the 0.90 significance level is not entirely precise, but it is clear that the fit of the data with the mathematical model is excellent. Although the goodness-of-fit test is a relevant and important test of any model, it does not exclude the possibility that alternative models might also be consistent with these data. The calculated confidence intervals indicate that for the class of models considered here, the parameters are precisely determined.

**DISCUSSION**

This mathematical model, like most models, requires a number of assumptions which are open to discussion and further biological testing. Our first assumption that the presence of ER in a metastatic site predicts a response rate ($Q$) which is close to 100% and was estimated mathematically as 93.3% is directly testable, if subtotal biopsies are done so that the same tissue can be monitored for response and assayed in vitro. Unfortunately, the majority of our biopsies were excisional biopsies, so this is not testable in our material.

We appreciate that factors other than the presence of the ER protein, such as progesterone receptor, may influence the response to hormone therapy. The effect of these factors is encompassed in the parameter $Q$ in our model. Further delineation of the role of these factors will not negate the basic concepts proposed here, unless they can be shown to predict 100% for response to hormonal therapy based upon examination of a single lesion for each patient. At least for progesterone receptor, this is not true.

One may question whether decreasing response with increasing disease extent is a general biological phenomenon and not a support of the proposed model. It is noteworthy that response to chemotherapy does not fall off with increasing number of sites of involvement over the range from 1 to >3 sites (7) or 1 to 5 sites. In another series (15), response rate appears to be lower ($p = 0.07$) for 3 or more sites versus one site, predominantly due to the impact of visceral involvement on response to chemotherapy rather than to number of sites. Thus, although the tissue is not settled, current data suggest that the observed inverse correlation between response rate and number of sites is related to the biology of response to hormone therapy, probably via clonal effects, rather than to nonspecific mechanisms.

A tacit assumption of our model is that $Q$ is not different for different organs of involvement. This is supported by observations that visceral involvement does not affect hormonal response. In a recent series, patients with ER+ tumors and visceral-dominant disease had a response rate of 61%, as compared to a 63% response rate observed in patients with ER+ tumors and soft tissue- and bone-dominant disease (3). Similar data have been reported by Manni et al. (10). These authors reported similar response rates to the antiestrogen, tamoxifen, in patients with visceral-, osseous-, or soft tissue-dominant disease.

Additional support for a clonal concept derives from the results of ER assays before and after endocrine therapy. In a series of 8 patients whose tumors were ER+ before endocrine therapy, repeat assays at a median of 11 months later were ER− in 4 patients and had fallen by 72 to 92% in the other 4 patients (1). These results suggest that endocrine therapy serves to accelerate the appearance of sublines which have a relatively low ER level.

The mathematical model proposed herein and the results of ER assays before and after endocrine therapy illustrate an important point about human tumor biology which has been labeled clonal evolution (13). As the cell population within a tumor expands, genetic instability permits the evolution of subpopulations with differing biological characteristics such as different ER status. In the absence of selection pressures, these clones may give rise to metastases with different ER status which underlies our mathematical model of response. When selection pressures are applied, as with endocrine therapy, endocrine-unresponsive clones will emerge.

The proposed model may be refined and extended through additional analyses. An example of a possible refinement of the model is incorporation of knowledge of the quantitative value of the ER result. In our 8 patients whose ER results were not concordant, the positive value tended to be low compared to that of patients in whom ER results were concordant and positive (11). Thus, for patients with a low positive ER value, a $P$ value of lower than 0.914 may be entered into the model, while for patients with very high ER values, $P$ higher than 0.914 may be appropriate. Additional data are needed to precisely predict $P$ as a function of the quantitative value of ER positivity.

A binomial model with the same $P$ for all intrinsically ER+ and ER− patients was used here as the simplest representation of concordance among multiple tumor sites. Similarly, we have assumed independence of response with identical probability $Q$ for all ER+ lesions of all patients. More extensive data with ER assays on multiple (>2) sites of the same patient are needed to adequately test these assumptions. In the absence of data demonstrating more complex associations, site dependencies, or interpatient differences, these assumptions appear to be a reasonable and simple basis for explaining the observed results. In our calculations, we have also used a value of 0.5 for the prior probability $(H)$ that an unexamined primary tumor is ER+. In general, this parameter could be made dependent upon menopausal status of the patient and could be estimated simultaneously with $P$. The model may also be applied when the biopsy is from the primary tumor rather than from a metastatic site, as in our data.

We encourage other investigators to evaluate our proposed hypothesis with regard to their data.

**REFERENCES**

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