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Predicting Treatment Response in Breast Cancer using Urinary Discriminant Ratio and Receptor Assay*

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Abstract—*The ratio of urinary 11-desoxy-17-oxo steroids to urinary corticosteroids (discriminant ratio, DR) was compared with levels of tumour oestrogen (ER) and progesterone (PR) receptor in 169 patients with early breast cancer. Approximately 20% of patients had significant levels in all three tests and 20% were negative by all three assays. However, patient status by ER and DR assay was in disagreement in 50% of cases. Retrospective analysis of 78 treatment courses of endocrine therapy in patients with disseminated breast cancer showed that out of the three predictive tests only ER assays reliably distinguished responding from non-responding patients. The same result was observed in a subgroup of 20 patients undergoing adrenal suppression with aminoglutethimide. When tests were combined in pairs, no combination, including either ER and PR or ER and DR, showed improved discrimination between responding and nonresponding patients compared to that seen with ER alone. No predictive assay or combination of assays could usefully distinguish patients with a high likelihood of response to endocrine therapy.*

INTRODUCTION

A RANGE of techniques have been used to identify patients with metastatic breast cancer who are likely to respond to endocrine manipulation [1]. Since only one-third of unselected patients show objective remission with hormonal therapy, clinical and laboratory measures to identify patients who have a higher likelihood of response are obviously desirable. Two biochemical procedures in particular have undergone widespread evaluation for this purpose. First, the estimation of steroid hormone receptors in human breast cancer tissue [2, 3] and second, the assay of urinary androgen metabolites [4]. Although simultaneous measurement of these variables in patients with breast cancer has been reported [5], to date there has been no detailed correlation between the response to treatment and the results of the two types of predictive test. The present report describes the use of receptor assays and urinary steriod measurements for predicting response of breast cancer patients to hormonal manipulation and compares their effectiveness when used singly or in combination.

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MATERIALS AND METHODS

Patients

The frequency of positive results of receptor and urinary discriminant ratio measurements was estimated in 169 patients with early breast cancer who had both types of test performed. In addition, the case records of a further 60 patients with metastatic breast carcinoma who had received a total of 78 courses of endocrine therapy were examined for evidence of response to treatment. All except four patients were post-menopausal. Details of the patients and treatment courses are given in Table 1. Evidence of complete or partial objective remission of disease with a minimum duration of three months was sought according to the criteria of Hayward *et al.* [6]. Patients' records, X-rays, scans and photographs were reviewed

Table 1. *Details of patients with metastatic breast cancer receiving endocrine treatment*

Response of patients to hormonal treatment	*n*	Number pre-menopausal	Age (mean and range)	Type of treatment*					Principal site of disease			
				T	Ag	E	A	Ab	Skin and chest wall	Bone	Nodes	Other†
Responding	29	3	54.3 (31–77)	17	9	2	1	–	11	8	9	1
Nonresponding	49	1	53.5 (30–77)	22	14	4	6	3	13	18	10	8
Total	78	4		39	23	6	7	3	24	26	19	9

*T = tamoxifen (10 mg b.d.); Ag = aminogutethimide (500 mg b.d. and hydrocortisone 20 mg b.d.); E = oestrogen (usually ethinyloestradiol 100ug daily); A = androgens (fluoxymesterone 5 mg b.d.); Ab = ablative surgery (1 hypophysectomy, 2 oophorectomy).
†Liver = 3; Lung = 6.

by a panel, including an external referee, and the results of the predictive tests were not disclosed during the assessment of response.

Predictive tests

A urinary steroid discriminant ratio (DR) was measured by the technique of Thomas *et al.* [7]. This measurement is the ratio of urinary 11-desoxy-17-oxo steroid metabolites divided by the level of urinary corticosteroid metabolites. A DR equal to, or greater than, 0.14 was considered to indicate an increased likelihood of response to endocrine therapy [8]. Measurement of DR was considered valid only if performed on patients aged 65 or younger, and was usually performed up to 6 months before endocrine therapy, although in a few instances up to 12 months elapsed between DR assay and treatment.

Steroid receptors for oestradiol (ER) and progesterone (PR) were measured in metastatic tumour tissue by a dextran–charcoal assay, as described previously [9]. In brief, frozen tumour was pulverised to a fine powder, thawed and homogenised in five volumes of assay buffer [9]. A cytosol preparation was obtained by high-speed centrifugation and aliquots were incubated with increasing concentrations of [^{3}H]-promegestone or [^{3}H]-moxestrol (progesterone and oestrogen receptor assay respectively). After 16 hr incubation at 4°C, bound and free labelled hormone were separated using dextran–charcoal [9], and binding sites per mg cytosol protein calculated by Scatchard analysis. A significant level of ER was taken as equal to or greater than 5 fmol/mg cytosol protein. At, or above, these levels the frequency of response to endocrine therapy using the current assay appears significantly increased compared with receptor-negative patients [3]. Receptor assays were carried out before any systemic treatment was given and within six months of treatment in all except 18 patients, who received two sequential treatments. In these patients receptor assays had been carried out between 3 and 16 months (mean, 6 months) before the second treatment.

Statistical analysis

The frequency of response in different patient groups was compared by Fisher's test.

RESULTS

The relationships between receptor status and DR analysis obtained on the same patients is shown in Table 2. Significant levels of ER and PR were found in 60% and 40% of patients respectively, and slightly over half of the patients had a positive discriminant ratio. Approximately half of the ER-positive group also had positive measurements of PR or DR. One-fifth of the group had all assays positive and a similar proportion were negative for all tests. It is of interest that the DR and ER tests were in disagreement in about half of the patients and that the percentage agreement between ER and DR status was very similar to that between PR and DR.

The relationship between the result of the individual types of predictive test and the objective response of patients to endocrine therapy is shown in Fig. 1. It is apparent that

Table 2. *Relationship between ER+ and ER− tumours, PR+ and PR− tumours, and patients with positive (DR+) or negative (DR−) urinary discriminant ratios. All expressed as percentage of total* (n = 169)

ER+ 57%	PR+ 39%	DR+ 54%
ER− 43%	PR− 61%	DR− 46%
ER+PR+ 34%	PR+DR+ 24%	DR+ER+ 30%
ER+PR− 23%	PR+DR− 15%	DR+ER− 24%
ER−PR+ 5%	PR−DR+ 30%	DR−ER+ 27%
ER−PR− 37%	PR−DR− 31%	DR−ER− 19%
ER+PR+DR+ 21%	ER−PR−DR+ 19%	ER−PR+DR− 1%
ER+PR−DR+ 10%	ER+PR+DR− 12%	ER−PR−DR− 18%
ER−PR+DR+ 5%	ER+PR−DR− 14%	

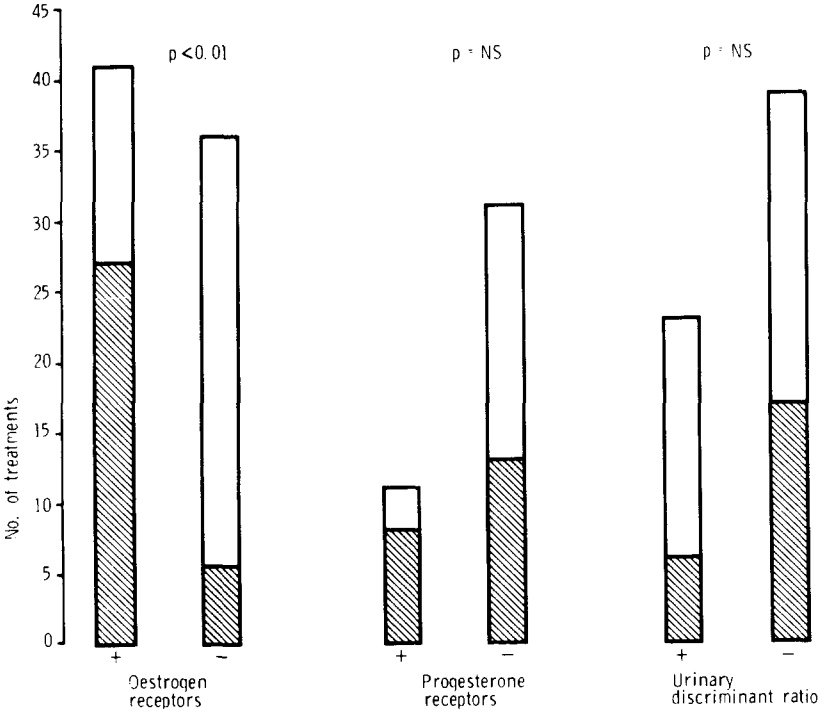

Fig. 1. Receptor status and urinary discriminant ratio measurements in breast cancer patients receiving endocrine treatment. Shaded area = patients responding to treatment.

only measurement of ER provided a significant discrimination between patients responding to hormonal manipulation and those who showed no response to treatment.

It was possible to estimate the value of receptor and DR estimations used in combination as a means of distinguishing hormone-responsive from non-responsive patients (Table 3). Although numbers in some categories are small it is clear that neither the combination of DR or PR results with ER estimations improves the predictive power of the ER results. Approximately 66% of patients with ER-positive tumours responded to endocrine therapy (Fig. 1), whereas 70% of ER+PR+ patients and only 43% of ER+DR+ patients responded. It is apparent from Table 3 that a response to endocrine therapy in ER-negative patients was confined to the subgroup who were also DR-negative. Of the three ER-negative patients who responded to endocrine therapy only one had a positive test by any other assay (ER−PR+). This patient was pre-menopausal and may have had a significant amount of nuclear ER with consequent PR synthesis [10]. Unfortunately, patient numbers were too small to permit analysis of subgroups of ER/PR/DR categories used together.

In view of the disappointing results obtained using DR analysis the data was reassessed after

redefining a positive DR result as equal to or greater than 0.12 (see Materials and Methods). There was still a failure to distinguish between hormone responsive and non-responsive patients (12/39 DR+ patients responded to treatment, 12/24 DR− patients responded to treatment, P = NS). An attempt was also made to restrict the DR results to a more defined group of patients who were less than age 60 and who did not have visceral disease. Again, no significant difference emerged between responding and non-responding patients (4/16 DR+ patients responded to treatment, 10/24 DR− patients responded to treatment, P = NS). Since DR measurements were initially described to select patients likely to respond to adrenalectomy or hypophysectomy [4], the response rates for "medical" adrenalectomy using adrenal blockade with aminoglutethimide in the present study were considered separately (Fig. 2). Whereas ER assays provided a significant discrimination between responding and non-responding patients, DR measurements did not distinguish between the two groups.

DISCUSSION

The management of metastatic breast cancer has been greatly influenced by the development of effective regimens of chemotherapy which may provide objective remissions in over 50% of patients [11]. It is thus becoming increasingly important to distinguish the subgroup of patients with disseminated breast cancer likely to respond to endocrine therapy, since delay in starting chemotherapy while awaiting a possible response to hormonal manipulation is unacceptable. In particular, a delay in patients who prove unresponsive to hormones may permit an increase in tumor bulk to an extent where chemotherapy may be much less effective. It must be remembered, however, that remissions obtained with hormonal therapy usually provide a good quality of life and are usually achieved with comparatively little risk of toxicity to the patient. It thus remains important to distinguish patients who are likely to respond to this form of treatment. Additionally, the advent of oral agents for endocrine manipulation which have relatively low toxicity, such as tamoxifen and aminoglutethimide, has added further impetus to the use of endocrine treatment. Measurements of tumour ER concentration have been found to identify a group of receptor-negative patients who have a poor likelihood of response to hormonal treatment [2]. However, there still remains a major difficulty in accurately predicting patients likely to respond to hormonal therapy, since approximately one-third of receptor-positive patients are also resistant to endocrine manipulation. Thus, the present study was undertaken to investigate the possibility that combining ER assay measurements with results from other predictive tests (PR and DR assays) might improve the ability to identify hormone-responsive patients more accurately.

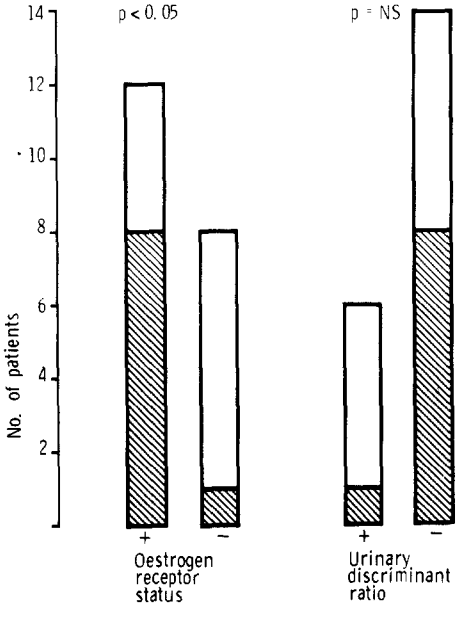

Fig. 2. Response of patients with metastatic breast cancer to treatment with aminoglutethimide and hydrocortisone. All patients had measurements of both oestrogen receptor and urinary discriminant ratio. Shaded area = patients responding to treatment.

It is clear from the data of Table 3 that a combination of DR and PR results with ER estimations does not improve the discriminating powers of ER measurement used alone. This is particularly disappointing in the case of PR assay since it has been suggested that PR synthesis in breast cancer may identify those tumours which have a biological response to oestrogen, and who would thus be likely to respond to endocrine treatment. Indeed, a number of papers have appeared claiming that ER+PR+ tumours have a significantly higher likelihood of response to hormonal therapy than ER+PR− tumours [10]. Not all have agreed with these findings [12] and PR assays were not found useful in the present study. However, patient numbers for combined ER/PR analysis were small (Table 3). Additionally, assay of PR is more difficult than ER [13] and measurements may be more open to laboratory error. This latter observation may explain some of the discrepant observations on the use of ER

Table 3. Relationship between response of patients to endocrine therapy and ER, PR and DR status

Patient group	Number responding / total number treated	Patient group	Number responding / total number treated	Patient group	Number responding / total number treated
ER+ PR+	7/10	ER+ DR+	6/14	PR+ DR+	1/2
ER+ PR−	11/15	ER+ DR−	15/21	PR+ DR−	5/7
ER− PR+	1/1	ER− DR+	0/9	PR− DR+	2/8
ER− PR−	2/16	ER− DR−	2/18	PR− DR−	6/9

and PR assays in combination referred to above [12].

The lack of improvement in ability to predict hormone-responsive patients by combining DR and ER assays might have been expected from consideration of the results seen with DR estimations alone (Fig. 1). It is apparent that DR measurements were unreliable when used in an attempt to identify patients responding to a range of different types of endocrine therapy (Fig. 1) or, more specifically, to aminoglutethimide and hydrocortisone treatment alone (Fig. 2). The reason for this failure to discriminate between hormone-responsive and non-responsive patients is unclear, since previous workers have found the measurements clinically useful for predicting the response to major ablative surgery [4]. Also, an earlier analysis of a small number of patients using the current DR assay showed apparent discrimination between responding and non-responding patients [8]. This discrepancy could in part be explained by the rigorous objective criteria used in the present study for determining treatment response [6]. Alternatively, there may be some fundamental biological difference between surgical adrenalectomy and the use of aminoglutethimide and hydrocortisone, which makes DR measurements unreliable for predicting response to pharmacological adrenal suppression.

In conclusion, the present results offer no support for the expectation that combining biochemical indices of tumour endocrine responsiveness will lead to better prediction of treatment response. It appears that ER assay remains the most reliable index to predict lack of response to hormonal therapy. By comparison, accurate means to predict hormone-responsive tumours is yet awaited.

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