

The differing predictive values of oestrogen receptor assays for large breast cancers

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Summary: Thirty elderly patients with T3 or T4 breast cancer underwent a wedge biopsy for radioligand-binding assay (RLA) of oestrogen receptor (ER) activity. A second, separate group of 21 elderly patients with T3 and T4 breast cancers underwent fine needle aspiration biopsy (FNA) for immunocytochemical assay of ER (ER-ICA). All the women received tamoxifen as primary treatment and response was assessed by UICC criteria. Tumour ER concentration by RLA was correlated with both response (Spearman's $R = +0.52$) and time to progression ($R = +0.76$). Nine patients with receptor-rich tumours (> 100 fmol/mg protein) failed to show a response. However, the percentage of cells staining for ER by ER-ICA assay was much more strongly related to the likelihood of response ($R = +0.89$); no patient with $< 20\%$ cells staining responded. Wedge biopsy and the biochemical determination of ER activity is of limited value in predicting the likely response to tamoxifen; ER-ICA assays on such tumours may be more informative.

Introduction

The primary treatment of elderly women with tamoxifen is becoming an increasingly popular form of treatment.¹ It is well tolerated by a group of patients who may prefer to avoid operation and who frequently have concurrent diseases, which increase the risks at surgery. However, recent studies have suggested that a significant proportion of such women will fail to respond to treatment with tamoxifen and that they might benefit from alternative therapy.^{2,3}

The anti-oestrogen tamoxifen is thought to act through the oestrogen receptor.⁴ In younger women, measurements of oestrogen receptor activity on wedge biopsies have been shown to be particularly helpful in the selection of likely responders and avoidance of unnecessary persistence with endocrine treatment in those in whom response is unlikely.⁵ In elderly women, however, breast cancers are more likely to be oestrogen receptor-positive^{6,7} and endocrine-responsive than those occurring in women of younger age groups.^{6,8} Here we have shown that, for elderly women with breast cancer, a standard biochemical method for assessing oestrogen receptor activity is of limited value in predicting response to tamoxifen by comparison with the results of previously reported studies of immunocytochemical staining for the receptor.^{9,10}

Methods

Between 1980 and 1986, increasing numbers of elderly women were treated primarily with tamoxifen alone. Prior to treatment, a wedge biopsy was carried out in 30 such women (aged 70 years of age or over at presentation), with T3 (5) or T4 (25) breast carcinomas, so as to obtain histological confirmation of malignancy and define oestrogen receptor status. The response to tamoxifen was retrospectively assessed according to UICC criteria. Progression was defined by a 25% or greater increase in the size ('area') of tumour and response by a reduction in size of 50% or greater.¹¹

Oestrogen receptor assays were carried out by ligand-binding assay as described previously,^{12,13} care being taken that all the wedge biopsies were transferred rapidly to the laboratory on ice. A representative portion was selected for assay by an experienced pathologist, and prior to assay, a further 50 μm section was taken and fixed to provide histological confirmation that the portion assayed contained breast cancer.¹⁴

The second cohort of 21 elderly women with T3 and T4 tumours, presenting between 1986 and 1988 underwent FNA for diagnosis and for the immunocytochemical assay (ER-ICA, Abbott Laboratories, Maidenhead) of ER on cytospin preparations as previously described,¹⁵ prior to treatment with 20 mg tamoxifen daily. At least 20 cells (and preferably greater than 100) per slide were con-

sidered necessary for assessment; aspirates with too few cells or uncertain pathology were considered unsatisfactory and excluded.¹⁵ For the present study, we have used a 'cut-off' of $\geq 20\%$ cells staining for receptor protein to indicate a 'clinically significant' level of receptor activity. These women were a sub-set of those on whom we have reported previously.⁹

The non-parametric Spearman's Rank correlation test was used to compare assay results with clinical response.

Results

Of the 30 patients whose tumours were assayed biochemically and who were treated by tamoxifen only, two were oestrogen receptor-negative (< 5 fmol/mg protein, that is, 93% oestrogen receptor-positive). Seven contained moderate to high amounts of oestrogen receptor (20–100 fmol/mg protein), and 21 were oestrogen receptor-rich (≥ 100 fmol/mg protein). Figure 1a shows the

initial result (progression, stasis or response) of treatment with tamoxifen in relation to the oestrogen receptor concentrations measured in the tumour biopsies. Twelve (40%) of the tumours (median ER value 46 fmol/mg protein) progressed, 14 (47%, median ER value 334 fmol/mg protein) responded to treatment with tamoxifen (although nine of these subsequently progressed), and four tumours (median ER value 251 fmol/mg protein) remained static (follow-up 11–70 months, mean 44 months). There was a statistically significant relationship between the oestrogen receptor concentration and the initial response to treatment (Spearman's Rank correlation coefficient $R = +0.52$, $P = 0.0028$).

In contrast, when the percentage of cells stained for ER by immunocytochemical staining in the second group of large T3 (4) and T4 (17) tumours was compared to response (Figure 1b) there was a much stronger correlation (Spearman's $R = +0.89$, $P < 0.0001$) and all nine of the patients showing tumour progression had low levels of staining ($< 20\%$ cells staining).

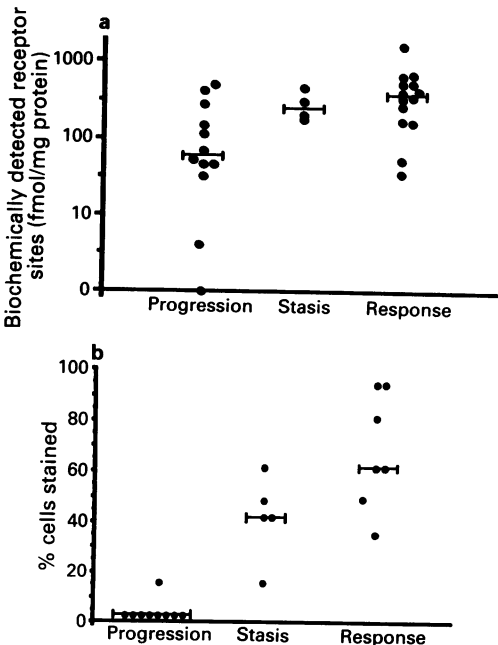


Figure 1 Relationship between the response to tamoxifen and oestrogen receptor activity in T3 and T4 tumours from elderly patients. (a) ER assessed by RLA assay on wedge biopsies ($n = 30$). (b) ER assessed by ER-ICA assay ($n = 21$). There were significant correlations between the ER concentration (Spearman's $R = +0.52$, $P = 0.0028$) and the percentage of cells stained (Spearman's $R = +0.89$, $P = 0.0001$) and the response to tamoxifen. $\bar{\quad}$ = median values.

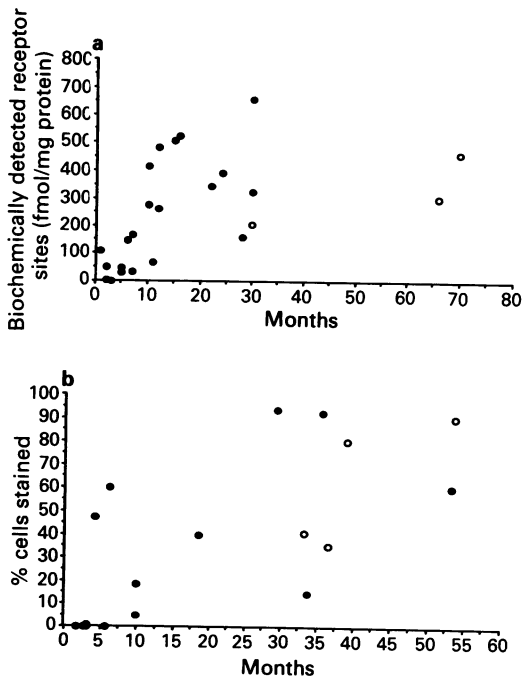


Figure 2 Oestrogen receptor concentrations of biopsies plotted as a function of time to clinical progression in patients with T3/T4 tumours. (a) Patients undergoing wedge biopsy and RLA (Spearman's Rank correlation coefficient $R = +0.76$, $P = 0.0003$). (b) Patients undergoing FNA and ER-ICA (Spearman's Rank correlation coefficient $R = +0.80$, $P = 0.0003$). Data for seven patients who achieved long-term disease control are also shown (O) but not included in the statistics because these tumours have still not progressed.

The oestrogen receptor status also correlated well with the time taken to clinical progression (Figure 2a and b). Twenty-four of the patients undergoing wedge biopsy were assessable for the time to progression (21) or duration of long-term stasis (3), and there was a strong correlation (Spearman's Rank correlation coefficient $R = +0.72$, $P = 0.0001$; Figure 2a). The remaining six patients died of other causes in the intervening years. For the second cohort of women who underwent FNA and ER-ICA assay, the percentage of cells staining for ER also correlated well with time to progression (Figure 2b; Spearman's Rank correlation coefficient $R = +0.80$, $P = 0.0003$). The abilities to select patients for the appropriate treatment by these two methods are compared in Table I.

Discussion

Classically, oestrogen receptor assays are carried out on portions of tissue obtained from primary breast cancers at the time of definitive surgery and then the results are subsequently correlated with the outcome of adjuvant therapy or the treatment of metastatic disease. The prognostic/predictive value of oestrogen receptor measurements has been variable and has failed to live up to initial expectations. Hähnel *et al.*,¹⁶ for example, reported that after 2 years, the prognostic significance of oestrogen receptors tends to diminish and, when one considers the heterogeneity of breast cancers, it is perhaps not surprising that assays carried out some time before correlate poorly with clinical response. In the present work, we have demonstrated that for a group of elderly women with larger and consequently more heterogeneous tumours, oestrogen receptor concentration, as determined by radioligand-binding assay immediately prior to treatment, was significantly related to the patient's

response to tamoxifen but the correlation was modest (Spearman's $R = +0.52$; Figure 1a). By an alternative method of assessment (time to progression), however, a stronger correlation (Spearman's $R = +0.76$) was observed between 'prognosis' and oestrogen receptor concentration.

Although these results do demonstrate the influence of ER status upon response to tamoxifen, the value of performing a wedge biopsy and radioligand-binding assay for ER appears to be limited; 24% (5/21) oestrogen receptor-rich tumours progressed without evidence of response. The ideal assay would have a point below which no tumours responded to tamoxifen and above which all tumours responded. In younger women, oestrogen receptor concentrations above and below 20 fmol/mg protein have proven to be significant predictors of the likelihood of response to endocrine therapy, with oestrogen receptor-rich tumours (> 100 fmol/mg protein) being the most likely to respond.^{5,17} Although elderly women tend to have breast cancers with higher concentrations of oestrogen receptor^{6,7} and are more likely to respond to endocrine therapy in general⁶ or to tamoxifen in particular,⁷ prediction of response by the above criteria (receptor levels) was less satisfactory. While in retrospect, two patients with ER-negative/poor tumours (< 20 fmol/mg protein) could have been spared ineffective treatment, there was no clear cut-off point to decide treatment with 28/30 (93%) patients having moderate/high levels of oestrogen receptor and 5/13 (39%) patients with oestrogen receptor-rich tumours (> 100 fmol/mg protein) failing to respond.

By comparison, selection for treatment according to the proportion of cells stained immunocytochemically was far more effective. There was a very strong correlation between the percentage of cells stained and either response or time to progression (Spearman's $R = +0.89$ and $R = +0.80$, respectively); a response rate of 64% was found for

Table I Comparison of relationships of 'ER status' by radioligand-binding and ER-ICA assays to response to tamoxifen in elderly patients with breast cancer

ER 'level'	Progression	Stasis	Response	% Response
Wedge biopsy and DCC assay (present work, $n = 30$)				
< 5 fmol receptors/mg protein	2	–	–	0
5–19	–	–	–	–
20–100	5	–	2	29
> 100	5	4	12	57
			Overall response rate 47%	
FNA biopsy and ER-ICA assay (modified from Gaskell <i>et al.</i> , 1989, ⁹ $n = 21$)				
0% cells staining	6	–	–	0
1–19% cells staining	3	1	–	0
$\geq 20\%$ cells staining	–	4	7	64
			Overall response rate 33%	

tumours with more than 20% of cells staining and all tumours with no evidence of immunocytochemical staining for oestrogen receptor progressed (Table I). The difference between the predictive capacities of these two types of assay could be partly a function of the 'cut-offs' used but, on the basis of the data presented, this seems unlikely. The ability of a FNA to sample a wider area of a heterogeneous tumour may provide a more representative biopsy and enhance the ability of the ER-ICA assay to predict response. Ideally we would have preferred to carry out these assays in parallel on the same group of patients. This was not possible, though we hope that such data will eventually be available from studies currently being undertaken by the department.

We conclude that by virtue of the simplicity of fine needle aspiration biopsy and the extra information concerning heterogeneity provided by the Abbott ER-ICA assay, the technique reported here

of wedge biopsy and biochemical assay is not cost-effective in selecting elderly patients for treatment with tamoxifen. It seems likely that these considerations will also apply to the use of the newer alternative form of the biochemical assay, the enzyme-immuno assay (ER-EIA), which yields apparently higher values for oestrogen receptor concentration than does the ligand-binding assay used here.

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