17 cases were positive by ICA but negative by DCC assay, although 5 showed some ER-activity in the DCC assay but this was below the >10 fmol/mg protein cut-off level. 52 cases were negative by ICA but positive by DCC assay; 17 of these 52 (33%) did show some ER-immunoreactivity but the H-score was well below 30. Agreement between the two methods was moderate (kappa = 0.537).

4. Conclusion

We found only a moderate concordance between ICA and DCC analyses of ER measurement in breast cancer tissue. If we change the golden standard from DCC to ICA, 23% of our breast cancer patients would have got a different therapy, if one limits hormonal therapy to patients with ER-positive breast cancer tissue. The ultimate usefulness of ER status assessment by ICA, however, resides in its ability to predict clinical outcome, especially response to hormonal therapy. It is clear that further studies are required to determine the predictive value of ICA.

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How best to express oestrogen receptor activity

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Abstract

Oestrogen receptor (ER) activity, detected and expressed in a variety of ways, is important in breast cancer. Experience in Edinburgh (1973–1996) showed that [1] no single mode of expression was entirely satisfactory, [2] the probability of a good 'outcome' (prognosis or response to endocrine therapy) increased with increasing activity (either fmol ER sites/mg protein or per cent cells staining for ER). Thus the use of a single 'cut-off' should be avoided and activity quantified, or stratified into categories.

Keywords: ER; Biochemical assay; Immunocytochemical assay; Response; Quantitation; Cut-off

1. Introduction

The oestrogen receptor (ER) protein, best established of the biological factors relating to 'outcome' in breast cancer, has been detected by a variety of biochemical methods and expressed as, e.g. fmol binding sites/mg protein or /μg DNA, or after immunocytochemical assay (ICA) as, e.g. per cent cells staining for ER, or 'histoscore'. Here, I review our experience in Edinburgh to help assess how best to express ER.

2. Methods

2.1. Patients and assessment of outcome

Five studies are considered:

1. A prospective study of 215 patients with operable disease followed up at 4–6 monthly intervals and by annual mammography [1].
2. Premenopausal patients with advanced disease, treated by surgical oophorectomy.
3. Patients with large (>4 cm), operable tumours, pretreated, after wedge biopsy, by neoadjuvant therapy.

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4. Elderly (> 65 years) patients treated, after fine needle aspiration (FNA) biopsy, with primary tamoxifen.
5. Elderly patients pretreated, after wedge biopsy, with neoadjuvant tamoxifen for 3 months.

2.2. Assays for ER activity

1. Radioligand-binding assay using dextran-coated charcoal absorption (DCC);
2. Enzyme immunoassay (ER-EIA, [1]); and
3. Immunocytochemical assay (ER-ICA) on frozen sections and on fine needle aspirates.

3. Results and discussion

3.1. Correlations between assays

Correlations ranged from excellent (Spearman's $R = +0.96$ for EIA versus DCC, on cytosol, $n = 63$) through good ($R = +0.87$ for ERICA per cent cells staining on frozen sections versus DCC on fresh tumours, $n = 34$) to modest ($R = +0.73$ for ERICA per cent cells staining in an FNA versus DCC on fresh tumours, $n = 56$, and $R = +0.60$ for ER staining on paraffin sections versus DCC on fresh tumours, $n = 100$). Thus, not all methods and modes of ER expression necessarily provide the same predictive information.

3.2. Heterogeneity

DNA content corrects biochemical values for variations in cellularity to some extent but protein content is simpler, a measure of cellularity [2] and almost universally adopted. Here, immunostaining methods though less quantitative, have a considerable advantage.

3.3. Relationship to prognosis

In a prospective study [1], ER concentration was the most important of the factors studied after 3 years.

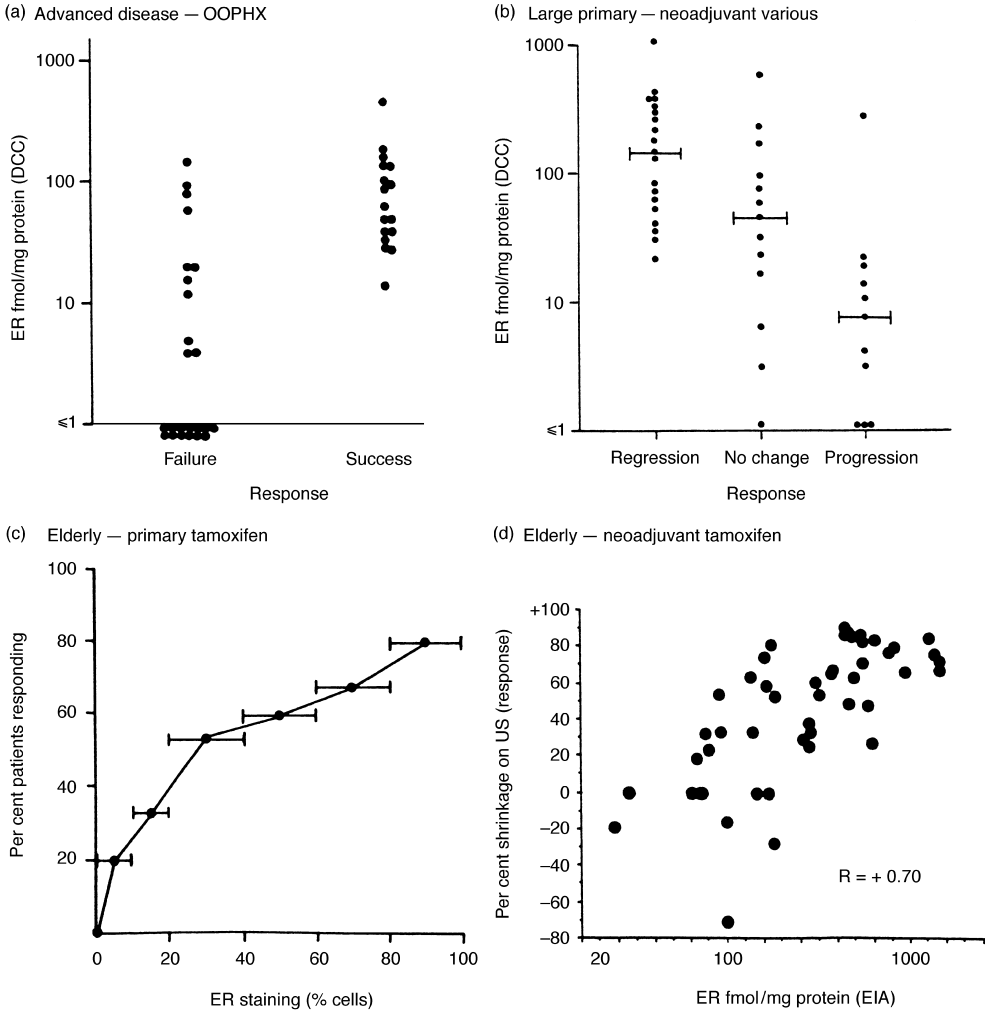

Fig. 1. The quantitative relationship between oestrogen receptor (ER) and response in (a) 41 premenopausal patients with advanced disease (response = International Union Against Cancer (UICC); (b) 43 patients with large, primary tumours (response by serial measurements); (c) 106 elderly patients (response > 25% diameter reduction by serial measurements); and (d) 48 elderly patients, ER > 20 fmol/mg (response by monthly ultrasonography).

After 6.6 years, ER concentration remains quantitatively related to risk of death or recurrence ($P < 0.0001$ by multivariate analysis).

3.4. Relationship to endocrine response

In premenopausal patients with metastases (Fig. 1a) and patients with large, operable tumours (Fig. 1b), those with no detectable ER failed to respond; median ER levels were much higher in responders.

In elderly patients treated with tamoxifen, the proportion responding rose with per cent cells staining for ER (Fig. 1c) and in further, selected (for significant ER) patients, the magnitude of tumour shrinkage correlated directly (Spearman's $R = +0.70$, $P = 0.0001$) with tumour ER concentration (Fig. 1d).

3.5. Possible reasons for moderate correlations with outcome

Heterogeneity and poor assays/specimen control may limit this correlation. With improved quality control and specimen care, endocrine response is $< 3\%$ in 'ER-negative' tumours [3]; here (Fig. 1), it was zero per cent. In addition, the strict criteria for response (UICC) are not attained by some clearly responding tumours [4].

4. Conclusions

Biochemical measurements (fmol/mg protein) are quantitative but cytochemical assays are cheaper and identify the cells staining. As previously noted [5]: (1) ER values form a continuum; (2) most breast cancers are hormone-sensitive to some degree; and (3) the probability of response to endocrine therapy increases with increasing ER level.

Quantitation or categorisation of ER values is, therefore, essential. The proposed 4-category scoring system for ICA by the UK Receptor Group (see [6]) is, thus opportune.

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