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ESTROGEN RECEPTOR IN PRIMARY BREAST CANCER ESTIMATED IN PARAFFIN-EMBEDDED TISSUE

A study of its usefulness compared to dextran-coated charcoal assay

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Abstract

Estrogen receptor (ER) was estimated immunohistochemically in formalin-fixed and paraffin-embedded tissue from the primary breast cancer in 349 postmenopausal patients with a high risk of recurrence and compared with the results of dextran-coated charcoal assay. There was a highly significant correlation between the ER classification obtained by the two methods ($p < 10^{-6}$). Patients ER positive according to immunohistochemical estimate had a significantly longer disease-free survival ($p < 0.001$) and survival ($p < 0.001$) than ER negative patients. The DCC assay showed an advantage of ER positive patients of the same magnitude. The patients, who were followed for a median of 86 months, were a subset of 1 700 patients participating in the Danish Breast Cancer Cooperative Group's randomized trial of adjuvant tamoxifen (TAM) treatment. In the presently analyzed subset of patients there were no statistically significant difference in disease-free survival ($p = 0.52$) or survival ($p = 0.54$) between patients who received adjuvant TAM and the controls. The same was true for receptor-defined subgroups regardless if the ER receptor was estimated in paraffin-embedded tissue or by the dextran-coated charcoal method. The analyzed subset might have been too small for demonstrating a positive effect of adjuvant TAM treatment.

Key words: Estrogen receptor, breast cancer, immunohistochemistry, paraffin-embedded tissue, dextran-coated charcoal assay, prognosis, adjuvant treatment.

A method using monoclonal ER antibodies for immunohistochemical determination of ER (ER-PAR) in sections of paraffin-embedded tissue has recently been developed (1, 2). This technique has shown a good qualitative and quantitative correlation with conventional binding assays, but the sensitivity was slightly lower as ER was not always detected in tumors with low ER contents (10–100 fmol/mg cytosol protein) (1–4), and fixation and tissue processing are important factors in the preservation of immunoreactive ER (2). Clinical data have shown that ER-PAR is a good predictor of response to endocrine therapy of advanced disease (5). In addition, the prognostic value of ER-PAR has been found comparable to either DCC assay or immunohistochemical assay performed on frozen sections of primary tumours, but with the limited number of patients in the study ($n = 130$), we were not able to evaluate the potential value of ER-PAR in selecting patients who might benefit from adjuvant TAM treatment (4).

A number of studies indicate a beneficial effect of adjuvant tamoxifen (TAM) therapy in early breast cancer (6–11), and experience from the treatment of advanced disease suggests that the estrogen receptor (ER) status may be a criterion for selecting patients for adjuvant therapy. This is supported by observations in some studies that only patients with ER positive tumors benefit from adjuvant TAM treatment (9, 10, 12). From 1977 to 1982, the Danish Breast Cancer Cooperative Group (DBCG) conducted a randomized trial (DBCG 77C) comparing radiotherapy (RT) with radiotherapy and tamoxifen (RT + TAM) in 1 700 postmenopausal women with primary high-risk breast cancer and the trial showed significantly fewer recurrences among TAM-treated patients (13). The ER content was measured using a dextran-coated charcoal assay (ER-DCC) in 349 of these patients, and with a median follow-up time of 42 months the results showed that only patients with more than 100 fmol ER/mg cytosol protein in the primary tumor benefited from TAM therapy (14).

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Consequently ER-PAR has now been analyzed in all of these 349 biopsies, and the aim of the present study was to examine the clinical usefulness of ER-PAR relative to a conventional binding assay (DCC) in patients with early breast cancer.

Material and Methods

Patients

The patients included in the present study were a subset from the DBCG 77C protocols whose ER content had been measured at the time of operation. The design and follow-up program of the DBCG trials have been described in detail elsewhere (15). Postmenopausal patients with initially operable breast cancer and a high risk of recurrence (tumor >5 cm in diameter and/or positive lymph nodes and/or skin or fascial invasion) were included in the protocol. The primary treatment was total mastectomy and partial axillary dissection. Women were considered to be postmenopausal if menostasia had persisted for more than 5 years. Eligibility criteria for protocolled treatment were absence of any evidence of advanced disease as estimated from general physical examination, routine blood tests, chest x-rays, and bone x-rays or scintigraphy. Further criteria were absence of any other previous malignant disease and informed consent. All patients received adjuvant radiotherapy (RT) to the chest wall, the axillary and periclavicular areas with a CRE-value of 1 335 and were then randomized to either observation or adjuvant TAM 10 mg $\times$ 3 daily for 48 weeks. This protocol enlisted a total of 1 700 patients from August 1977 until November 1982, but due to a delay in the organization of nationwide receptor analyses, malignant tissue from the primary tumour from only 349 patients was sent for steroid receptor analysis. Patients with receptor analyses have previously been shown to be comparable to those without receptor data with respect to age, lymph node status, tumor size and adjuvant treatment (16).

The patients entered the study between August 1979 and November 1982. The clinical data were evaluated as of June 1, 1989 at which time the median observation period was 86 months. Disease-free survival (DFS) and survival (S) was calculated as the period from mastectomy until the date of recurrence or death (DFS) or death (S).

Steroid receptor analysis

ER-PAR. Immunocytochemical analysis of paraffin-embedded tissue was performed as previously described in detail (1, 2). The tissue used for this analysis was sections from the biopsy originally sent for routine receptor analysis. These tissue blocks had been fixed in 10% buffered formalin for 24 h and stored at room temperature up to 9 years before the immunohistochemical analysis. The tumors were classified as ER positive or ER negative according to criteria previously described (1, 2). In brief, a tumor was classified as ER-positive if any degree of specific nuclear staining recognizable above control was seen. Evaluation of staining features was done without knowledge of clinical or ER-DCC data. The specificity of the monoclonal ER antibodies used has previously been documented (17–19). The ER-PAR assay failed in 40 tumors, leaving 309 patients with an ER-PAR status. The failures were mainly due to inadequately fixed biopsies.

ER-DCC. The ER content was measured by a DCC assay as recommended by the EORTC (20) and with the modifications previously described (21) for 323 of the patients. Tumors were considered ER positive if there was at least 10 fmol/mg cytosol protein. In all cases the receptor content was measured in the primary tumor and in histologically verified malignant tissue. The DCC assay failed in 12 tumors, leaving 311 patients with an ER-DCC status. The failures were mainly due to technical faults (trays dropped on the floor, etc.).

Statistics

Estimation of DFS and S was performed by life-table analysis and the significance of differences between selected groups was assessed by log-rank analysis (22). Estimation of the relative risk in selected patient groups was performed as suggested by Crowley (23). Analysis of contingency tables was performed using Fisher's exact test or the χ^2-test.

Results

Patient characteristics. The characteristics of the patients are shown in Table 1. The prognostic variables were evenly distributed between the two treatment groups.

Comparison of assays. Using a cut-off limit of 10 fmol/mg cytosol protein the ER-DCC assay detected a higher frequency of ER positivity and classified 244 of 311 tumors as positive (79%) while ER-PAR classified 231 of 309 tumors as ER positive (75%). This difference was not statistically significant ($p = 0.28$). Statistically there was a highly significant association between the ER classification obtained by the two assays and the classifications agreed in 79% of the tumor specimens (Table 2). The disparities were due to 35 tumors (13%) characterized as ER-DDC positive/ER-PAR negative and 22 tumors (8%) classified ER-PAR positive but ER-DCC negative. The ER-DDC positive/ER-PAR negative tumors occurred among tumors with intermediate concentrations of ER (median 37 fmol/mg cytosol protein).

Comparison of assays and clinical parameters. Using either assay, ER positive patients were observed to experience both a longer DFS and S (Fig. 1). The survival curve of ER-DDC positive patients was very similar to that of

Table 1

Patients' characteristics

Characteristic	p-value	RT n (%)	RT + TAM n (%)
Total number		176	173
Age (years)	0.79		
<70		127 (72)	128 (74)
⩾70		49 (28)	45 (26)
Tumor size (cm)	0.47		
0–2		35 (20)	27 (16)
3–5		72 (41)	80 (46)
>5		69 (39)	66 (38)
Positive nodes (number)	0.22		
0		28 (16)	27 (16)
1–3		88 (50)	101 (58)
>3		60 (34)	45 (26)
Degree of anaplasia	0.84		
I		39 (23)	34 (21)
II		98 (58)	96 (58)
III		33 (19)	35 (21)
ER-DCC	0.80		
Positive		121 (78)	123 (79)
Negative		35 (22)	32 (21)
ER-PAR	0.58		
Positive		114 (73)	117 (76)
Negative		42 (27)	36 (24)

Table 2

Relationship between immunohistochemical and biochemical ER assays in human breast tumors

ER-PAR	ER-DCC (fmol/mg cytosol protein)		
	Positive*	Negative	Total
Positive	182	22	204
Negative	35	38	73
Total	217	60	277

* ER-DCC positive defined as ⩾ 10 fmol ER/mg cytosol protein. $p < 10^{-6}$ (χ^2-test).

ER-PAR positive patients as were the survival curves of the ER-PAR negative and ER-DCC negative patients. The survival advantage of ER positive patients was quantitated by estimation of their relative risk (Θ) compared to ER negative patients. The results (Table 3) confirmed the picture displayed by the survival curves, viz. a reduced risk of suffering an event (recurrence or death) of 0.5 if the primary tumor was ER positive. There were no statistically significant differences between the assays, and the results suggest that overall the ER assay in paraffin-embedded tissue is equivalent to the ER-DCC assay for prognostic purpose. Fig. 2 shows the prognostic significance of discordant assays compared with concordant assays. Patients classified as ER positive by both assays carried the best prognosis, patients who were negative by both assays

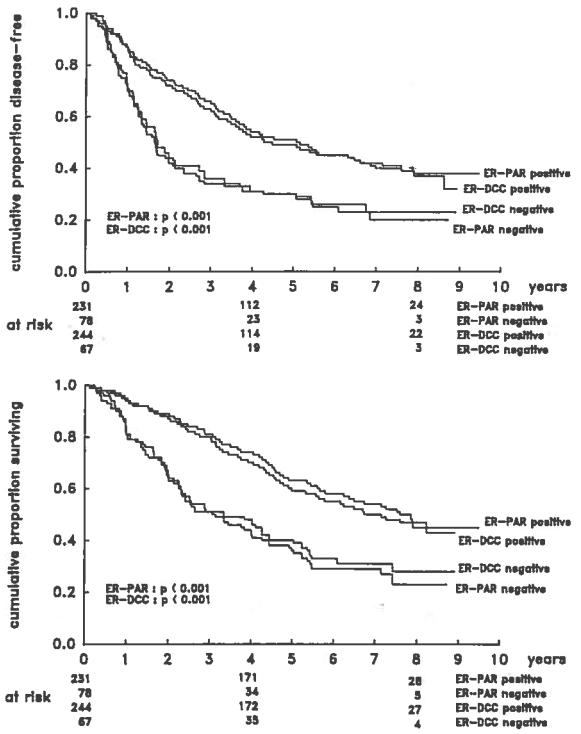

Fig. 1. Disease-free survival (top), survival (bottom) and ER status. All patients. ER-PAR: Paraffin assay. ER-DCC: Dextran-coated charcoal assay.

carried the worst prognosis and patients with disparate assays had an intermediate prognosis.

Although adjuvant TAM treatment significantly improved DFS in the total population of 1 700 patients participating in the DBCG 77C study, the effect was not significant in the subpopulation analyzed in the present study. This is shown in Table 4. There was no significant difference between the group with adjuvant TAM and the control group concerning DFS ($p = 0.52$) or S ($p = 0.54$). The merit of ER assays for selecting patients to adjuvant TAM treatment was studied by analyzing the effect of adjuvant TAM in the receptor-defined subgroups. Generally, a benefit of adjuvant TAM treatment could be observed among ER positive patients, but this advantage did not reach statistical significance in any subgroup. We have previously reported on a benefit of adjuvant TAM in patients with ER-DCC ⩾ 100 fmol/mg cytosol protein (median follow-up 42 months). A reanalysis of this patient group shows that the benefit has diminished below statistical significance at the present follow-up time of 86 months (Table 4). The patients classified positive by both assays had the best prognosis as shown in Fig. 2 and might therefore be the patients benefiting most from adjuvant TAM treatment. Estimation of DFS and S in this subgroup is also shown in Table 4. The group treated with adjuvant TAM had a small although non-significant DFS advantage but did not have any S advantage.

Table 3

The estimated relative risk (RR) by ER status for all patients irrespective of treatment. RR = relative risk of patients with ER positive tumors

Assay	Number pos/neg	Disease-free survival			Survival		
		RR	c.l.*	p-value	RR	c.l.*	p-value
ER-PAR	231/78	0.50	0.38–0.71	0.00003	0.43	0.32–0.63	$<10^{-6}$
ER-DCC	244/67	0.56	0.40–0.77	0.001	0.53	0.37–0.77	0.0001

ER-PAR: Paraffin assay; ER-DCC: DCC assay; *: 95% confidence limits.

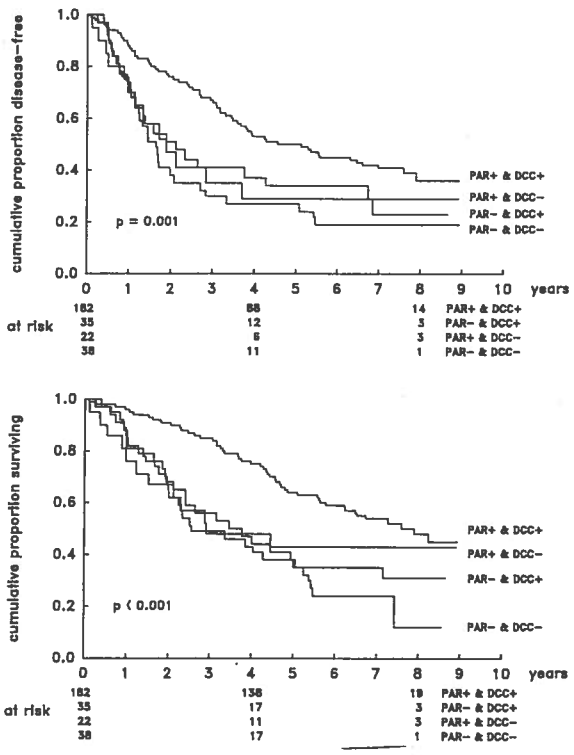

Fig. 2. Disease-free survival (top) and survival (bottom) for patients with similar and disparate ER assays. ER-PAR: Paraffin assay. ER-DCC: Dextran-coated charcoal assay.

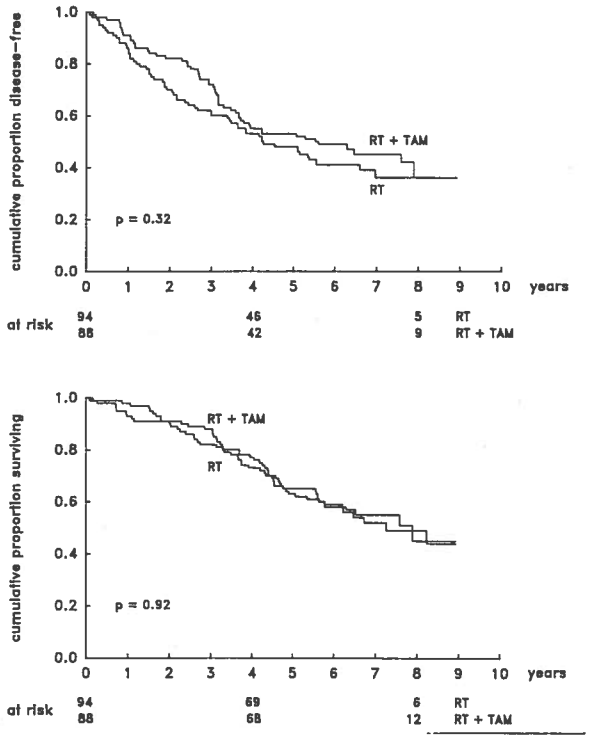

Fig. 3. Disease-free survival (top), survival (bottom) and adjuvant treatment. Patients with tumors classified as ER positive by both ER assays. RT: Radiotherapy. RT + TAM: Radiotherapy and tamoxifen.

Discussion

The paraffin assay was slightly less sensitive than the DCC assay, as also demonstrated in previous studies (1–3). The lower sensitivity can be explained by the fixation process which causes a loss of immunodetectable ER (2). The 'false negative' tumors have a low ER content (typically < 100 fmol/mg protein), which diminishes the clinical significance of the sensitivity loss. The overall agreement between the ER-DCC and the ER-PAR assays was in the present study of the same magnitude as reported from comparisons between ER-DCC and immunohistochemical ER analyses in frozen sections (24–26). Several tumors were classified as positive by the ER-PAR assay but negative by ER-DCC. This is partly explained by the rather arbitrary cut-off level of 10 fmol/mg cytosol protein for defining a tumor as ER positive. Some of these tumors do contain ER positive tumor cells, but the ER concentration in the cytosol is diluted below the cut-off level.

The present study showed that ER-PAR determination in formalin-fixed tissue sections from the primary tumor provided a prognostic information on DFS and S comparable with that of an ER-DCC assay in fresh tissue. Our observation of the role of ER as a prognostic parameter in primary breast cancer is consistent with the observations made by many others (8, 27–30).

We could not detect any significant effect of adjuvant TAM treatment in the patients studied here even though they constituted a subgroup of the patients in the DBCG 77C protocol in whom an overall beneficial effect of adjuvant TAM has been reported (13, 16). The patients studied

Table 4

The estimated relative risk (RR) of patients treated with RT + TAM versus patients treated with RT alone

Parameter	Assay	Stratum	RT/RT + TAM n	RR	Confidence limits*	p-value
Disease-free survival						
	None	All pts.	176/173	0.91	0.71–1.25	0.42
	ER-PAR	ER−	42/36	1.70	1.00–3.33	0.07
		ER+	114/117	0.71	0.59–1.25	0.24
	ER-DCC*	ER−	35/32	0.91	0.59–2.00	0.14
		ER+	121/123	0.91	0.67–1.25	0.34
	ER-DCC	ER ⩾ 100	75/77	0.67	0.43–1.11	0.07
	Both	PAR+/DCC+	94/88	0.82	0.56–1.29	0.32
Survival						
	None	All pts.	176/173	0.91	0.71–1.25	0.54
	ER-PAR	ER−	42/36	1.43	0.91–2.50	0.19
		ER+	114/117	0.91	0.63–1.43	0.54
	ER-DCC*	ER−	35/32	0.77	0.45–1.43	0.35
		ER+	121/123	1.00	0.67–1.43	0.89
	ER-DCC	ER ⩾ 100	75/77	0.67	0.45–1.11	0.10
	Both	PAR+/DCC+	94/88	0.98	0.66–1.60	0.98

* ER-DCC positive defined as ⩾ 10 fmol ER/mg cytosol protein.
DFS: Disease-free survival; S: Survival; RT: Radiotherapy; RT + TAM: Radiotherapy and tamoxifen; ER-PAR: Paraffin assay; ER-DCC: DCC assay; *: 95% confidence limit.

here have previously been shown to be comparable to the patients in the main study in terms of major prognostic variables (14). The reason for the lack of any effect of adjuvant TAM in this study could therefore be ascribed to the number of patients studied. Thus, assuming a 50% risk of an event a hypothetical experiment with two treatment groups of equal size would demand a total of 700 patients in order to trace a 10% difference between the groups.

Stratification of the patients according to ER status suggested that the effect of adjuvant TAM treatment was of the same magnitude in ER-PAR positive and ER-DCC positive patients with high ER contents, but the advantage did not reach statistical significance in either of the groups. We have previously reported a statistically significantly prolonged DFS in ER-DCC positive patients with high ER levels at a time when the median follow-up was 42 months (14), but the difference has evidently narrowed below statistical significance in the present study with a median follow-up of 86 months. This could speak in favor of prolonging adjuvant TAM treatment beyond one year as suggested by the NATO trial (8).

The Chemotherapy Consensus Conference has recommended stratification of patients for adjuvant hormonal treatment according to their ER status (31). This is in accordance with the general experience in hormonal treatment of advanced disease and is supported by several studies showing that only patients with ER positive tumors benefit from adjuvant TAM treatment (9, 10, 12). The picture is not clear-cut, as shown in the present study, and in addition, a couple of large studies has shown the effect of TAM to be independent of ER status (7, 8). The recent overview published by the Early Breast Cancer Trialists' Group concluded that a trend exists towards a greater relative effect of tamoxifen with a greater ER level, but ER analyses did not seem to be able to pick out patients wholly unresponsive to tamoxifen (32). Some of the problems of interpreting the relation between ER and the treatment effect are undoubtedly secondary to the fact that only a fraction of the patients in the relatively large studies mentioned above had their ER measured (DBCG: 20% (14) Scottish: 43% (7), Nato: 41% (8)). Unfortunately, all commonly applied receptor assays (based on steroid binding assays or monoclonal antibody technology) demand fresh tissue which makes a retrospective collection of the required data impossible. The problems could be solved in two ways: First, one could await the result of newer studies with a higher frequency of receptor determinations. Second, one could try to apply a method utilizing paraffin embedded tissue. The results of this study indicate that the paraffin ER assay used here may at least partly solve the problem.

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