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Short Recurrence-free Survival Associated with High Oestrogen Receptor Levels in the Natural History of Postmenopausal, Primary Breast Cancer

Susan M. Thorpe, Ib. J. Christensen, Birgitte Bruun Rasmussen and Carsten Rose

The ability of oestrogen and progesterone receptor (ER and PgR, respectively) status to discriminate recurrence-free survival (RFS) among a cohort of consecutively accrued 952 postmenopausal patients has been studied. None of the cohort members investigated were treated with adjuvant therapy. Using a graduated scale of receptor status [low, intermediate and high receptor levels (< 10 vs. 10–107 vs. ≥ 108 fmol/mg cytosol protein, respectively)] instead of the more commonly used dichotomous subdivision (positive vs. negative), ER level significantly discriminated between groups of patients with long vs. short RFS. Contrary to our expectations, patients with highest ER levels have as poor a prognosis as ER-negative patients, while patients with intermediate ER levels have longest RFS. The group of patients with ER levels ≥ 108 fmol/mg cytosol protein comprises 47% of the cohort. The independent significance of overexpression of ER as a prognostic factor among this patient group is demonstrated in multivariate analysis where ER level is more significant than either grade of anaplasia or tumour size. PgR status did not significantly predict RFS among these patients. While the highest ER levels predispose for poorer prognosis among postmenopausal patients, it is precisely this group that experiences *greatest* benefit from adjuvant treatment with tamoxifen. Thus, patients who might otherwise go untreated due to their node-negative status can be readily identified and offered adjuvant treatment.
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INTRODUCTION

WHILE THE majority of node-negative, primary breast cancer patients have normal life expectancies following primary surgery and do not, therefore, require adjuvant therapy, a significant proportion (20–30%) [1] will experience recurrent disease. A recent review [2] has reiterated the need to define the prognostic factors that enable distinction between these two groups of patients. Despite numerous investigations, the role of receptor status has not yet been fully clarified. Nevertheless, it has become dogma that oestrogen receptor (ER)-positive patients fare better than ER-negative patients. This paper scrutinises that belief and finds it untrue in the case of a significant, readily identifiable and biologically relevant subset of the patient population.

In the nationwide Danish Breast Cancer Cooperative Group (DBCG) trials for treatment of primary breast cancer, receptor analyses have been performed since 1979 for as many patients as possible. The design of the DBCG trials facilitates studies of the natural history of breast cancer: approximately half of the patients were evaluated to be at such a low risk of recurrent

Correspondence to S.M. Thorpe.
S.M. Thorpe is at the Department of Tumor Endocrinology, The Fibiger Institute of the Danish Cancer Society, Ndr. Frihavnsgade 70, 2100-DK, Copenhagen Ø; I.J. Christensen is at the Finsen Laboratory, University Hospital, Strandboulevarden 49, 2100-DK, Copenhagen Ø; B.B. Rasmussen is at the Department of Pathology, Roskilde County Hospital, Kogevej 7–13, Postbox 247, 4000-DK, Roskilde; and C. Rose is at the Department of Oncology R, Odense University Hospital, Sdr. Boulevard 29, 5000-DK, Odense C, Denmark.
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disease (tumour size < 5 cm, no lymph node metastases, no fixation of the tumour to skin or fascia) that no adjuvant treatment is given subsequent to primary surgery.

When describing ER and progesterone receptor (PgR) concentrations in the DBCG studies in relation to the natural history of breast cancer, certain features are clearly associated with patient age and/or menopausal status. The observed, significant increase in ER level is, for example, significantly associated with both age and menopausal status [3]. Similarily, the less common receptor profiles of ER−PgR+ and ER+PgR− characteristically occur among women of different menopausal status: the profile of ER−PgR+ occurs almost exclusively among premenopausal patients, while the profile of ER+PgR− occurs predominantly among postmenopausal patients [3]. Similar observations have been made in other large patient populations [4, 5].

The natural history of recurrence-free survival (RFS) for receptor-positive (≥10 fmol/mg cytosol protein) vs. receptor-negative patients also differs in the two menopausal groups. Premenopausal receptor-positive patients have significantly longer RFS than receptor-negative patients, while no difference is discernable between the two groups for the postmenopausal patients [6]. Meanwhile, in patients treated with adjuvant therapy which, by definition, affects the natural history of the disease, RFS is significantly longer for ER-positive than for ER-negative patients in both menopausal groups [7, 8].

The puzzling observation that ER positivity is associated with longest RFS for all patient groups *except* the postmenopausal group receiving no adjuvant therapy prompted a more scrupulous study of receptor status in relation to RFS for this group of patients.

As stated above, it is common knowledge that ER concentrations are significantly higher among post- than premenopausal patients. Earlier we have shown that while the distribution frequency curve for receptor concentrations appears to be normal for the premenopausal patients, it is significantly skewed toward higher values for ER in the postmenopausal group [3]. Implementing a graduated scale of receptor status (low, intermediate and high receptor levels, −, + and ++, respectively) instead of a dichotomous subdivision (positive vs. negative), ER level is found to be a highly significant and independent prognostic factor in the natural history of postmenopausal breast cancer (i.e. patients *not* treated with adjuvant therapy). Unexpectedly, patients with the highest ER concentrations were found to have the poorest prognoses.

PATIENTS AND METHODS

The patients in the present study are from the DBCG protocols for treatment of primary breast cancer. The organisation, design and follow-up of the DBCG program have been described in detail elsewhere [9, 10]. In accord with the entrance criteria for the most recent DBCG protocols, only women <70 years of age were included in the present study. In the DBCG program women are classified as postmenopausal when menostasia has persisted for at least 5 years. If the patient does not fulfil the criterion for being postmenopausal but is 50 years of age or older, she is considered perimenopausal. The remaining women are classified as premenopausal.

The following criteria were evaluated for patients eligible for entry into adjuvant protocols at the time of the primary operation and were used to distinguish between patients at low or high risk for recurrent disease: (a) whether there is lymph node involvement; (b) whether the tumour is >5 cm in diameter; and (c) whether there is tumour invasion in overlying skin and/or deep fascia.

If none of these criteria were present, the patient was considered to be at low risk for recurrent disease and received no further treatment subsequent to surgery. High-risk patients all received some form of adjuvant therapy. To put the present findings into perspective, it has been necessary to briefly examine RFS in relation to ER status in several of the DBCG protocols for high risk patients. Relevant treatment arms are described in the appropriate figure legends.

Routine follow-up of DBCG patients consists of clinical examinations at regular, predefined intervals for 10 years [9, 10].

Within the geographical area serviced by the Copenhagen receptor laboratory, all protocolled, postmenopausal patients who fulfilled the above criteria and who had histologically verified malignant tissue in the biopsy forwarded to the receptor laboratory between September 1979 and September 1989 are included in the present study. Thus, this study encompasses approximately one-third of all Danish primary breast cancer patients registered during the specified time period. To ascertain the representative nature of the 952 patients comprising the study cohort, features of patients with and without ER assays are shown in Table 1.

Death/recurrence data were evaluated as of 1 September 1989. Recurrence is defined as the appearance of new lesion(s) in patients with no previous evidence of disease, as confirmed by physical examination, biopsies and/or other relevant diagnostic procedures. Cause of death is not recorded; the frequency of deaths due to causes other than breast cancer is assumed to be equal in different patient subgroups for patients of the same age. Median time of observation, defined as the median value for the time between the primary operation and the date of evaluation, is 48 months.

Receptor analyses

Tissue from primary breast cancer tumours was analysed for ER and PgR concentrations in a single laboratory using the dextran-coated charcoal method for multipoint titration analysis recommended by the EORTC [11] with the minor modifications previously described [12]. The laboratory performed continuous quality control studies of the ER and PgR assays in collaboration with other European laboratories in the EORTC receptor group. Using a cut-off level of 10 fmol/mg cytosol protein, 80 and 66% of the 952 postmenopausal patients were classified, respectively, as ER-positive and PgR-positive.

The dissociation constant (K_d) for binding between ER and oestradiol was evaluated as previously described [13].

Statistical methods

The SAS program [14] was used for data base management, descriptive statistics, and life-table analysis. Cox analysis [15] was performed using the BMDP program [16].

Deconvolution of ER concentrations [expressed as log (ER+1)] into two normal distributions was done by maximum likelihood assuming a constant coefficient of variation analogous to an algorithm presented by Vindeløv and Christensen [17].

The comparisons of characteristics between groups of patients were performed by standard χ^2 tests in the relevant contingency tables. Two-sided P values < 0.05 were considered significant.

RESULTS

The representative nature of the subset of patients with ER analyses has been studied in relation to the entire population of

Table 1. Characteristics of low risk, postmenopausal patients with and without ER assays

Variable	*P* value	With ER assay *n* (%)	Without ER assay *n* (%)
Total no.		952	2270
Age (years)	0.08		
40–49		10 (1)	43 (2)
50–59		317 (34)	832 (37)
60–69		615 (65)	1395 (62)
Tumour size (cm)*	<0.01		
≤1		107 (11)	482 (21)
2		406 (43)	980 (43)
3		270 (28)	480 (21)
4		90 (10)	150 (7)
5		26 (3)	70 (3)
>5		5 (1)	5 (0)
Not recorded		48 (5)	103 (5)
No. nodes removed	0.74		
1–3		212 (22)	519 (23)
≥4		732 (77)	1726 (76)
Not recorded		8 (1)	25 (1)
Grade of anaplasia†	0.21		
I		314 (38)	648 (39)
II		397 (48)	818 (49)
III		123 (15)	204 (12)
Clinical status (as of 1 September 1989)	0.16		
Recurrent disease/dead		230 (24)	497 (22)
Receptor status‡			
ER positive (n=952)		759 (80)	
PgR positive (n=719)		473 (66)	

*Tumour size evaluated pathoanatomically: clinical tumour size was utilised as an entrance criterion for the protocol 77-la patients. Pathoanatomical tumour size is not recorded for 48 of these patients, but the clinical size ranges from 1 to 5 cm for these 48 tumours.
†Ductal carcinomas only.
‡Receptor positivity defined as ≥ 10 fmol/mg cytosol protein.

DBCG patients without receptor analyses (Table 1). Large tumours are overrepresented among patients with receptor analyses in relation to those without analyses, reflecting the fact that tissue from larger tumours is more often sent for receptor analysis. In all other respects (e.g. age, number of lymph nodes removed, grade of anaplasia, and clinical status), the two populations are similar.

Selection of limits defining low, intermediate, and high ER levels

The frequency distribution patterns of ER concentrations for pre-, peri- and postmenopausal primary breast cancer tumours are shown in Fig. 1a and b. While the patterns of the logarithmically transformed values of ER among pre- and perimenopausal patients are approximately normally distributed, the distribution of ER values among the postmenopausal patients is clearly skewed toward high values, as previously noted [3]. As seen in Fig. 1b, two normal distributions fit into the frequency distribution curve for the postmenopausal patients. The χ^2 test for goodness of fit indicates a reasonable agreement for this resolution (P = 0.1).

As is customary, the cut-off level of 10 fmol/mg cytosol protein constituted the boundary between low and intermediate receptor concentrations. The upper boundary (intermediate/ high) was set based on the fitted normal distribution shown in Fig. 1b. The value for twice the standard deviation from the mean of the first normal distribution of receptor values was employed, which corresponds to 108 fmol/mg cytosol protein. This cut-off level to distinguish between + and ++ ER levels is close to the arbitrarily chosen level of 100 fmol/mg cytosol protein previously implemented by us [7] as well as others [18–21] in other contexts.

Recurrence-free survival among low-risk postmenopausal patients

Univariate analysis of RFS for this group of patients demonstrates no significant difference (P = 0.48) between ER-positive and ER-negative patients using the customary cut-off limit of 10 fmol/mg cytosol protein, which is in accord with our previous finding [6]. However, using the more graduated subgrouping (− vs. + vs. ++) with the limits described above, a significant difference in RFS was observed (P = 0.04) (Fig. 2a). Surprisingly, the ER++ group has a prognosis as poor as that of the ER-negative group. While the statistically inferred level of 108 fmol/mg cytosol protein has been used to delineate between intermediate and high ER levels, even higher discriminatory power with regard to RFS is obtained using either 100 or 120

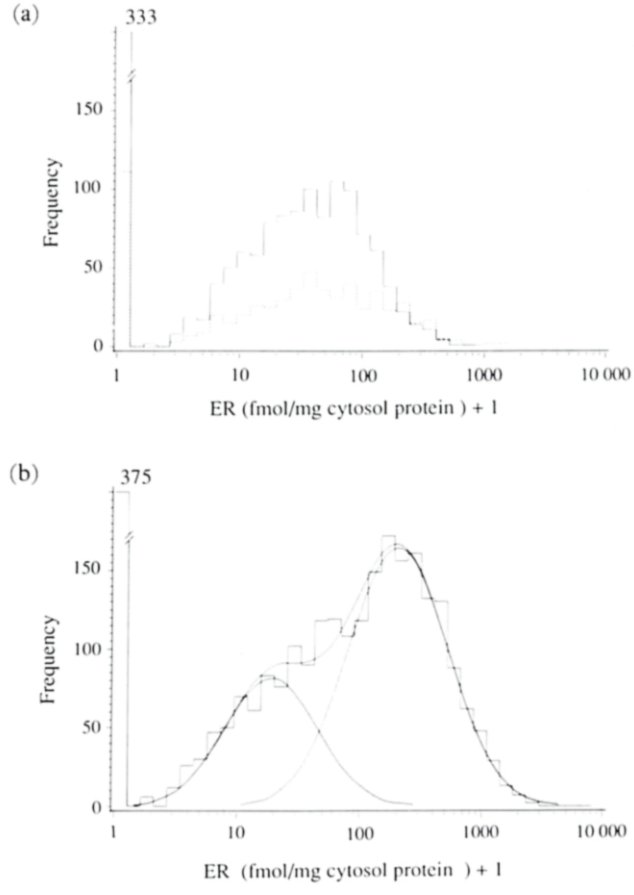

Fig. 1. Frequency distribution patterns of ER concentrations in primary breast cancer biopsies: pre- and perimenopausal patients (solid and broken line, representing 1444 and 611 patients, respectively) are shown in (a); postmenopausal patients (representing 2640 patients) are shown in (b). The two normal distributions that can be fit into the frequency distribution pattern for postmenopausal patients are indicated.

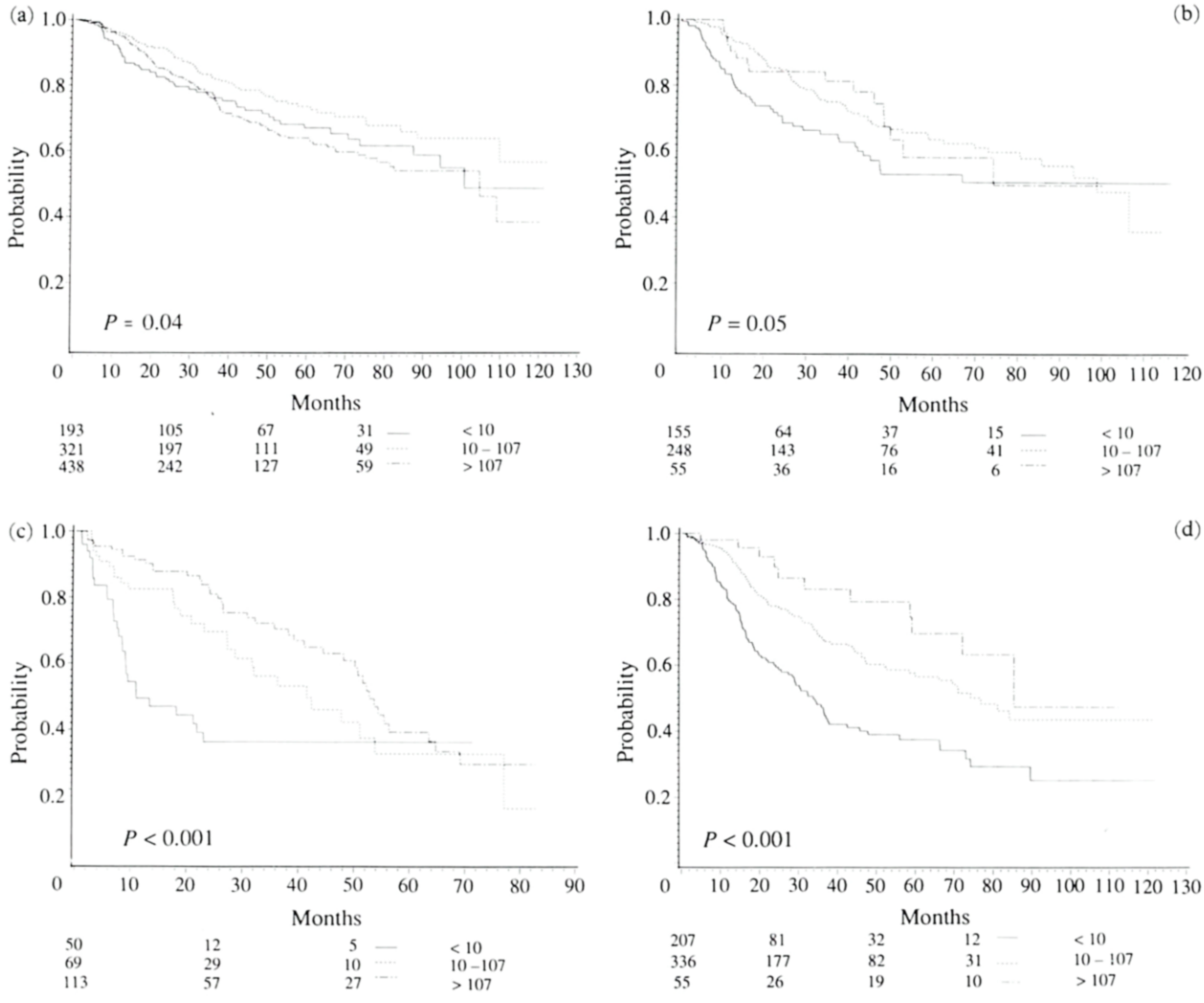

Fig. 2. RFS curves for postmenopausal (< 70 yr) (a and c) and premenopausal (< 50 yr) (b and d) patients not treated with adjuvant therapy (a and b, respectively) or treated with systemic adjuvant therapy (c and d, respectively). Low (< 10 fmol/mg), intermediate (10 fmol/mg ≤ ER < 108 fmol/mg), and high (≥ 108 fmol/mg) ER levels are indicated by solid, stippled, and broken lines, respectively. All patients in panel c received tamoxifen (TAM) therapy (1 year) as the sole form of adjuvant therapy. All patients in panel d received one of four adjuvant chemotherapy regimens [RT (radiation therapy) + C (cyclophosphamide); RT + CMF (cyclophosphamide, methotrexate, and 5-fluorouracil); CMF; or CMF + TAM]. The number of patients for each receptor level at 0, 2, 4, and 6 years is indicated below the abscissa.

fmol/mg cytosol protein (P = 0.03 and 0.02, respectively). Moreover, if the 298 perimenopausal patients (≥50 years, but not defined as postmenopausal) who were accrued during the same time period are included in the above univariate lifetable analysis, the P-value is 0.02; ER++ patients persist in having a RFS as poor as that of ER-negative patients (data not shown).

Since ER status is known to be strongly associated with tumour size, degree of anaplasia, and age [4, 6], multivariate analysis was performed to determine which prognostic factors are independently significant. A total of 778 postmenopausal patients were available for multivariate analysis encompassing these variables as well as ER level and number of lymph nodes removed (variables a–e, Table 2). Cox multivariate analysis eliminated tumour size and age from the model. Even when age was scored as a continuous function (logarithmically) in the multivariate analysis, it was not significant (P = 0.47 to enter). The variables retained as having independent prognostic value are those shown to the right in Table 2. ER level and number of lymph nodes biopsied are the most important of the prognostic factors.

In subsequent multivariate models, we tested for possible interactions between ER level and either PgR status or K_d value. The latter was investigated because of our previous finding that high K_d values for binding between oestradiol and ER are associated with poorer RFS for postmenopausal patients not treated with systemic adjuvant therapy [13]. Because age had no prognostic value in either univariate or the initial multivariate analysis, it was eliminated from these subsequent models, while all the variables with independent significant value in Table 2(a, c and e) were retained. The analysis of PgR status included 592 patients while that testing K_d level included 598 patients. Neither PgR status nor K_d level were found to be significant independent prognostic factors.

To insure that our finding did not result from an opportune choice of cut-off levels, the data was checked using quadratic regression analysis. Univariate quadratic regression analysis

Table 2. Variables included in analyses of RFS and their respective scorings for Cox analysis

Variable	Univariate analysis	Multivariate analysis				
	P-value Log rank test	Scoring	Variable excluded *P*-value to enter	Variable retained Beta	S.E.	*P*-value
(a) Number of lymph nodes removed (n=944)	0.005					
1–3		1				
≥4		2	Included	−0.42	0.16	0.008
(b) Tumour size (n=904)	0.07					
≤2 cm		1				
>2 cm		2	0.23	—	—	—
(c) Grade of anaplasia (n=834)	0.05					
I		1				
II		2				
III		3	Included	0.21	0.11	0.048
(d) Age (n=952)	0.52					
40–49		1				
50–59		2				
60–69		3	0.45	—	—	—
(e) Oestrogen receptor level (n=952)*	0.04					
Low		0 otherwise 1				
Intermediate		1 otherwise 0				
High			Included	−0.41	0.17	0.013
(f) Progesterone receptor status (n=719)	0.70					
PgR <10		1				
Pgr≥10		2	0.84	—	—	—
(g) K_d level (n=789)	0.26					
K_d<1.5		1				
K_d≥1.5		2	0.39	—	—	—

*<10 = low ER. 10≤ER<108 = intermediate ER. 108≤ER = high ER. In multivariate analysis, ER level is scored as a bivariate indicator variable. The values shown for the Cox analysis are obtained analysing low and high ER vs. intermediate ER.

found ER concentration highly significant in predicting RFS (n = 952, P = 0.01). Multivariate quadratic regression of RFS hazard for ER confirmed the significance of ER concentration maintained significance (n = 778, P = 0.02) along with grade of anaplasia and number of lymph nodes removed. Thus, our findings show RFS to be shortest among patients with both the lowest and the highest concentrations of ER.

Recurrence-free survival among other patient groups

In the three remaining patient groups [premenopausal with (i) or without (ii) adjuvant therapy, and postmenopausal patients treated with adjuvant therapy (iii)], subdividing ER levels into three (− vs. + vs. ++) groups rather than two (− vs. +) does not alter our previous reports that ER positivity is associated with longest RFS (Fig. 2b–d). In the natural history of the premenopausal group, patients with + or ++ levels of ER experience approximately the same RFS, and one that is significantly better than that seen for ER-negative patients (Fig. 2b). Interestingly, among patients treated with adjuvant therapy (Fig. 2c and d), those with ++ ER levels have an even better RFS than that seen for ER + patients. Figure 2b and d also re-emphasises the fact that few premenopausal patients actually have high ER levels; while the ER++ groups comprises approximately 47% of the postmenopausal population, it constitutes only 10% of the premenopausal group.

DISCUSSION

This is the first report demonstrating that ER level is a highly significant, independent prognostic factor for RFS among postmenopausal, primary breast cancer patients not treated with adjuvant therapy, i.e. for the natural history of the disease. The importance of ER level is equal to that of the accuracy of classification of the patients as truly node-negative, and supercedes that of grade of anaplasia. Although highly significant, the magnitude of the difference in RFS among these postmenopausal, untreated patients grouped according to receptor status is modest. The startling observation is the fact that patients with highest ER levels have a RFS as poor as that of ER-negative patients. While ER concentrations are positively correlated to age, it is highly unlikely that high age alone can account for the poor RFS observed in the group of ER++ patients. The variable of age was eliminated from the multivariate analysis and is, therefore, inferior to ER level with regard to prediction of RFS.

It is notable that this is not the first report that high ER levels among postmenopausal patients are associated with a prognosis as poor as that seen for ER-negative patients. This observation was first reported in 1983 in a small group of patients (n = 137) [18], but has largely been ignored. In a recent update of the Scottish Tamoxifen Trial patients, the 5-year recurrence rates for 280 postmenopausal patients allocated to the observation arm were 48 vs. 29 vs. 46% for patients with ER levels of < 20

vs. 20–99 vs. ≥ 100 fmol/mg cytosol protein, respectively (Dr H.J. Stewart, The Scottish Trials Office, Edinburgh). These data corroborate our present report.

ER status has not previously been shown to be a significant prognostic factor among postmenopausal patients not treated with systemic adjuvant therapy. Five published studies [6, 19–22] are large enough (patient number ≥450) and have sufficiently long times of observation (median time of observation ≥4 years) to permit addressing the question of whether ER status is associated with the natural course of the disease. Prolonged RFS is observed for ER-positive patients in four of the five investigations when the patient population is considered as an entity [6, 19–21]. In our own study [6], the overall benefit of ER positivity was found due solely to a highly significant difference observed only among the premenopausal patients. Although published studies have used different criteria to define menopausal status, two of the remaining four studies corroborate this finding [19, 20]. In contrast, one of the five finds a nearly significant difference in RFS ($P < 0.06$) only among postmenopausal patients [21]. The remaining study [22] that found no overall difference in RFS between ER-positive and ER-negative patients, is also unique in that frequency of ER positivity is very low (46% compared to 64–75% in the other four studies).

When evaluating the potential association between ER status and the natural course of the disease, two groups of patients are usually identified: those with little or no measurable ER vs. those with measurable ER levels. Cut-off limits vary from 3 to 20 fmol/mg cytosol protein in defining these two groups. More graduated receptor levels (low, intermediate and high levels) are occasionally considered, especially when evaluating the potential benefit of adjuvant endocrine therapy [7, 18, 20, 23]. As seen here, a difference in RFS according to ER level for postmenopausal patients will probably be observed in other large studies wherein graduated receptor levels are implemented. As was the case in our own data, dichotomous analysis does not reveal the difference.

Recognising that ER positivity is widely accepted as a hallmark of better prognosis in breast cancer, it is counterintuitive that higher levels of ER predispose for a poorer prognosis than intermediate levels of ER. An association between high levels of ER and high levels of thymidine kinase activity has previously been noted [24] and would tend to support the present finding. Experimental data obtained with breast cancer cell lines are also valuable in attempting to understand the biological mechanisms underlying our finding. Two independent groups have reported overexpression of ER in experimental breast cancer cell lines that have been adapted to growth *in vitro* in the absence of hormones [25–27]. Cell proliferation rate is observed to be higher in these cultures (a characteristic of more aggressive tumours); and, while the cells have escaped hormonal control of growth regulation, hormonal stimulation of specific proteins, e.g. PgR, is still possible [25–27]. These findings from *in vitro* experiments corroborate the clinical observations presented here: provided that they do not receive adjuvant therapy, patients with high (i.e. overexpressed) compared to intermediate concentrations of ER have a poor prognosis; nevertheless, the overexpressed ER appears to be capable of mediating a 'normal' receptor-mediated response, i.e. effect of tamoxifen observed (Fig. 2c); moreover, the overexpression of ER is observed predominantly among postmenopausal patients who have lower circulating levels of oestrogens.

It is presently unknown whether the higher levels of ER associated with poorer prognosis might reflect (a) mere overexpression of the same form of ER and/or (b) occurrence of an 'abnormal' or variant form of ER. Publications validating the occurrence of variant forms of ER appear in the literature with ever-increasing frequency [28–36]. Earlier experimental data from our laboratory indicate that an 'abnormal' form of ER might exist and that this putative 'abnormal' ER appears most frequently in the postmenopausal group [13, 37]. It may be significant that the distribution pattern of receptor concentrations among postmenopausal patients consists of at least two populations, one representing 'normal' and the other representing overexpressed concentrations of ER. Natural selection of a population of cells consequent to hormone deprivation—experimentally impinged as cited above, or as one of the normal physiological consequences of the menopause—could be the mechanism responsible for evolution of cells that overexpress ER.

Overexpression of ER may be a hallmark of tumour progression from a fully hormone-dependent to a hormone-independent state via a hormone responsive state. Later steps in tumour progression might entail loss of ability to express ER and/or PgR as well as the inability to mediate effects of anti-oestrogens. These concepts are in accord with a hypothesis suggested earlier by Dabre and King [38] based on observations in mouse mammary tumours.

Despite the association between poor natural prognosis and high ER levels, the DBCG data demonstrate that adjuvant systemic treatment with anti-oestrogens (tamoxifen) is associated with prolonged RFS for patients with both high and intermediate ER concentrations (Fig. 3c). This corroborates a recent report from the NSABP regarding node-negative patients [20]. Hence, even if overexpression of ER proves to be associated with occurrence of a variant form of ER, this potentially altered receptor apparently maintains the capacity to mediate the effect of tamoxifen such that adjuvant treatment remains possible.

This paper demonstrates that untreated postmenopausal patients with high levels of ER have a prognosis as poor as that of ER-negative patients. Identification of this subset of patients that comprises almost half of the entire low risk, postmenopausal group is possible by routinely performed ER analyses. Prospective clinical trials in which low risk, postmenopausal patients with high ER levels are randomised to either treatment with adjuvant tamoxifen or observation seem justifiable and timely. Meanwhile, we suggest that overexpression of ER among postmenopausal patients indicates tumour progression and may be associated with occurrence of a variant form of ER that retains the capacity to mediate the action of tamoxifen. Whether a higher frequency of variant forms of ER occurs among patients overexpressing ER is currently being investigated. Our data indicate that overexpression of ER is significant biologically and clinically. The observation that overexpression of ER is associated with poor RFS among untreated patients yet with excellent RFS among patients treated with adjuvant tamoxifen may be helpful in understanding the biology of the disease.

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