The interaction of oestrogen receptor status and pathological features with adjuvant treatment in relation to survival in patients with operable breast cancer: a retrospective study of 2660 patients

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Abstract

The oestrogen receptor (ER) status of 2660 patients with primary breast cancer has been related to the effect of different adjuvant systemic therapies on survival. However, as patients in the various treatment groups also had different prognostic features comparison between treatments was difficult. Over 90% of patients receiving tamoxifen (Tam) were postmenopausal compared with <20% of those receiving chemotherapy (CT). The latter had more positive nodes (85% vs 54%) and grade III tumours (54% vs 30%) than the Tam group. The combined CT and Tam group had similar characteristics to the CT alone group. The current reported increase in the proportion of women with ER+ tumours is explained by immunohistochemical analysis of ER and screening programmes. ER status was unrelated to survival in patients with small, low grade, node-negative tumours which was no different from that expected for age-matched women taken from the general population. The value of adjuvant treatment in these patients is therefore questionable. In those given any adjuvant treatment, survival of women with ER+ tumours was prolonged, with the greatest effect being seen in those receiving Tam. Patients with ER- tumours benefited from CT but the addition of Tam to CT improved survival only in those with ER+ tumours. ER status is now established as a major predictive factor for treatment selection in primary disease. Studies of prognostic and predictive markers may be invalidated by use of adjuvant therapy and selection criteria for different treatments. Survival will be influenced by both tumour biology and therapy. This important consideration must be remembered when analysing new markers, particularly in small studies.

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Introduction

Nowadays, primary treatment for the majority of patients with early breast cancer includes some form of adjuvant therapy in addition to surgery and radiotherapy. This means that studies of prognostic features are confounded by these additional treatments as they modify the course of the disease. The situation is further complicated by the criteria used to select adjuvant therapy for individuals, with the inevitable result that patients with different prognostic features receive different treatments. Thus, assessment of features that might predict response to specific adjuvant therapies may also be confounded.

The importance of the above and the resulting difficulty in assessing accurately both prognostic and predictive features in breast cancer is illustrated in the
current study. We have reviewed the significance of oestrogen receptor (ER) status in a large series of patients treated with different adjuvant regimes in a single centre over a 23-year period. The patients were all treated for primary breast cancer at the Guy’s Hospital Breast Unit between 1975, when the cytosol ligand-binding assay for ER was introduced, and the end of 1998 by which time immunohistochemical assays were used to determine receptor status.

Adjuvant chemotherapy (CT) was first used in the Unit in 1975 for the treatment of premenopausal women with node-positive breast cancer. Shortly afterwards adjuvant tamoxifen (Tam) was introduced for postmeno-pausal women with node-positive disease. Initially, adjuvant treatment was undertaken within prospective randomised clinical trials, (tamoxifen in a multi-centre trial (Baum 1985) and melphalan and cyclophosphamide, methotrexate and 5-fluorouracil in the Guys/Manchester trials (Rubens et al. 1983, Howell et al. 1984)) which have contributed to the overviews reported by the Early Breast Cancer Trialists’ Collaborative Group (1998a,b).

These studies have influenced the approach to adjuvant systemic treatment over the period of the work reported here. As the value of additional treatment became firmly established, trials with an untreated control arm were discontinued and the majority of patients in the current study received adjuvant treatment in accordance with prevailing practice at the time.

Following meta-analyses of published clinical trial data that confirmed the efficacy of adjuvant treatment this is now generally used in women with both node-negative and node-positive disease (Early Breast Cancer Trialists’ Group 1998a,b). This increased use of adjuvant therapy has had a major impact on survival (Beral et al. 1995); it has affected different prognostic groups to different extents, frequently diminishing the apparent effect of biological markers. This point should be recognised when assessing the relevance of new markers.

**Materials and methods**

The relationship between survival and ER status in patients who did not receive any adjuvant therapy and in those who received adjuvant therapy of any type was analysed, first as a whole and then in relation to individual treatment regimens. Within the treatment groups we further investigated whether or not the effect of adjuvant therapy was modified by tumour stage (size and nodal status) and grade of the primary ductal carcinomas.

**Patients**

A total of 2660 patients with known ER status of their tumours formed the study group. All had operable, stage I or II breast cancer and were treated at the Guy’s Hospital Breast Unit between 1 January 1975 and 31 December 1998.

**Treatment**

Patients were treated either by total mastectomy and axillary clearance (modified radical mastectomy) or by breast conserving therapy (tumorectomy with axillary clearance followed by radiotherapy to the preserved breast).

The cases were divided into 4 groups according to adjuvant treatment received: (1) No treatment, (2) Tam only, (3) multi drug chemotherapy (cyclophosphamide, methotrexate and 5-fluorouracil; CMF) and (4) sequential chemotherapy and Tam.

**Histopathological analysis**

Tumours were typed according to the World Health Organisation’s classification; infiltrating ductal carcinomas were graded using the criteria of Bloom and Richardson as modified by Elston and Ellis (1991). Tumour size was based on clinical measurement; axillary node involvement was determined by pathological examination of all nodes in the axillary clearance specimen (average of 25.5 nodes per specimen).

**ER measurement**

During the period of study the methodology changed as newer reagents became available. Initially ER status was determined by cytosol ligand-binding assay (King et al. 1979). In January 1987 it was replaced by the Abbott enzyme immunoassay (EIA), which was used until 1996 (Jensen et al. 1986). Originally, the cut-off value to distinguish ER+ from ER− tumours was 10 fmol/mg cytosol protein. This was changed to 20 fmol/mg following the introduction of the EIA, in accordance with guidelines issued by the European Organisation for the Research and Treatment of Cancer (EORTC) quality assurance group and Abbott laboratories, who supplied the EIA kits. Throughout this time (1976–1996) the percentage of ER+ cases was between 65 and 70%, in agreement with most other laboratories (Jensen & DeSombre 1993). As part of our internal quality control procedures, we carried out three-monthly checks on the proportion of ER+ and ER− cases and this remained relatively constant whilst we were doing cytosol assays. From 1996 ER status was assessed by an immunohistochemical (IHC) assay on formalin-fixed, paraffin-embedded tissue sections, using antibody ID5 (Al Saati et al. 1993). Although careful comparative studies were initially carried out between EIA and immunohistochemistry, we found that our percentage of positive cases...
increased with time. Initially, this may have been due to improvements in our IHC technique, but the proportion of ER + cases soon settled at around 75%. This sustained increase is almost certainly explained by the inclusion of many smaller, better differentiated tumours, which were too small to be analysed previously, but could now be assessed by the IHC assay on routine histological sections. Tumours showing any positive nuclear staining (weak, moderate or strong) in more than 10% of tumour cells were classed as positive. Throughout the period of patient accrual the laboratory took part in external quality assurance schemes (Rhodes et al. 2001).

Statistics
The distribution of ER status in the different treatment groups was studied through contingency tables and associated chi-squared tests, while the effects on survival of ER status and treatment were illustrated by Kaplan-Meier plots and tested with the log-rank test. A Cox’s Proportional Hazards model was undertaken on all of the data and clearly indicated significant complex interactions to warrant looking at individual treatment interaction components.

Results
The tumours from 1880 (71%) of the 2660 patients were ER positive and 780 (29%) were ER negative. The distribution of ER status of patients in the different treatment groups and the characteristics of the patients as a whole and for the 4 main treatment groups are shown in Tables 1 and 2.

Clinical and pathological features of patients in different treatment groups
Menopausal status data were missing from some 12% of patients overall; this figure was similar for each treatment group. Due to our treatment policy, the percentage of pre- and postmenopausal patients differed considerably between the various treatment groups. Only 8% of patients who received Tam were premenopausal compared with 82% of those who received CT alone and 71% who received CT plus Tam. Over 60% of patients in all groups had tumours measuring >2 cm.

More patients with involved lymph nodes received adjuvant treatment than did those with uninvolved nodes (67% compared with 35%). In the Tam-treated group 54% of the cases were node positive, compared with the 85% of cases that received CT alone and 84% who were treated with both CT and Tam. There was also a marked difference between the percentage of cases with grade III infiltrating ductal carcinoma; in the no treatment and Tam-treated groups 30% of cases were grade III, but in the two groups receiving CT this increased to over 50%. It is evident, therefore, that those women who received adjuvant CT, either alone or in combination with Tam, had tumours with poorer prognostic features, a factor to be taken into account when considering the outcome of individual groups.

Analysis of individual treatment groups
No adjuvant treatment
There were 1324 patients who were not given any adjuvant treatment. Some of them came from the control groups of the various trials of adjuvant treatment carried out in the earlier years of this study; for others adjuvant treatment was not deemed necessary in view of their favourable clinical and pathological prognostic features. The tumours from 893 (67%) patients were ER+ with 431 (33%) being ER−. With a median follow-up time of 11.5 years (max. 26.8 years) the log-rank test indicated that there was no evidence of any difference in survival attributable to ER status (Fig. 1). For the first 10 years, the patients with ER + tumours had a better chance of survival whilst after 10 years patients with ER− tumours appeared to have the better chance. Most of these patients had good prognostic features (node status, tumour size and histological type and grade) since those with poor prognostic features had been selected for adjuvant treatment. Nodal status ($\chi^2 = 270.29$, $P < 0.0001$), tumour size ($\chi^2 = 51.55$, $P < 0.0001$) and histological grade ($\chi^2 = 50.72$, $P < 0.0001$) each predicted outcome in the expected manner (data not shown). Further log-rank analyses were carried out to examine the interaction between ER status and these features. We found that the survival of patients, selected on clinical grounds to not receive any adjuvant treatment, depended on grade, nodal status and tumour size, but not on ER status. A series of Cox regression models found no significant interactions between node status and grade, or tumour size and grade or node status and tumour size. Thus the analysis showed that ER status is uninformative for patients who do not require adjuvant treatment. Patients with small (<2 cm), low grade tumours and node-negative disease had the best prognosis and their survival curve is not different from the averaged survival taken from the table of expected death rates for women matched for age from the general population, as shown in Fig. 2.

Any adjuvant treatment
In total, 1336 patients received some form of adjuvant treatment. There were 987 (74%) with ER + tumours and 349 (26%) with ER− tumours. Figure 3 shows the significantly better survival of women with ER + tumours via free access
who received adjuvant treatment as compared with the women with ER− tumours ($\chi^2 = 52.1$, $P < 0.0001$); the median follow up was 6.3 years (max. 22.1 years).

**Adjuvant tamoxifen**

Adjuvant Tam alone was used in 831 cases. As expected patients with ER+ tumours gained considerable benefit (Fig. 4). ER status was positive in 659 (79%) and negative in 172 (21%) tumours. After a median follow-up of 6.5 years (max. 22.1 years) patients with ER+ tumours had a better survival rate than those with ER− tumours (ER+ 91% vs ER− 71% survival after 5 years and 78% vs 60% survival respectively after 10 years) ($\chi^2 = 37.49$, $P < 0.0001$). The advantage for patients with ER+ tumours was the same, irrespective of tumour size ($\chi^2 = 56.5$, $P < 0.0001$) and nodal status ($\chi^2 = 103.5$, $P < 0.0001$) (data not shown).

**Adjuvant CMF**

Three hundred and fifty four patients received adjuvant chemotherapy alone. ER status was positive in 209 tumours (59%) and negative in 145 tumours (41%). Although patients with ER+ tumours treated with CMF did better than those with ER− tumours, the difference was not as great as that seen with Tam ($\chi^2 = 5.69$, $P = 0.0017$; Fig. 5). With a median follow-up of 6.7 years (max. 21.8 years), patients with ER+ tumours had a 5-year survival rate of 84% and a 10-year survival rate of 62% compared with 69% and 52% for the corresponding survival rates for those with ER− tumours. CT appeared to be of most benefit to patients with ER+ tumours, first when the tumours were under 2 cm (10-year survival: <2.0 cm ER+ 77%, ER− 55% compared with >2.0 cm ER+ 53% and ER− 52%, $\chi^2 = 14.5$, $P = 0.0022$) and secondly in those patients with positive lymph nodes (10-year survival: node-negative ER+ 75%, ER− 86%; node-positive ER+ 61%, ER− 44%, $\chi^2 = 18.05$, $P < 0.0004$) (data not shown).

**Adjuvant CMF and tamoxifen**

This combination treatment was introduced more recently for patients who on clinical grounds were thought likely
to benefit from the addition of Tam to CMF. The number of patients in this group is small and follow-up is not as long as for other groups. However, after a median follow-up of 5.5 years (max. 10.9 years), it appears that patients with ER+ tumours benefited from this combination therapy ($\chi^2 = 5.57, P = 0.018$: Fig. 6). Only 151 patients received the combined treatment; there were 119 (79%) with ER+ tumours and 32 (21%) with ER-/C0 tumours. Patients with ER+ tumours had a 5-year survival of 83% compared with 62% for those with ER-/C0 tumours.

Analysis of survival in relation to tumour size shows an advantage for patients with ER+ tumours irrespective of size ($\chi^2 = 10.3, P = 0.016$). Similarly, in the presence of nodal metastases there was also an advantage for those with ER+ tumours, the patients with ER-/ node-positive tumours having a particularly unfavourable prognosis ($\chi^2 = 12.5, P = 0.0058$) (data not shown).

Adjuvant CMF compared with no treatment

In order to evaluate the benefit of the different adjuvant treatments, we tried to compare their survival effects with that of a comparable group of patients who received no such treatment.

As the majority of patients in this study who received adjuvant treatment were selected on the basis of clinical grounds (menopausal status and pathological features) and were not part of a trial, it has proved impossible to find a suitably large control group with the same clinical profile for the tamoxifen-treated patients. However, 274 of the CMF-treated patients were treated within the Guy’s/Manchester CMF trial (Howell et al. 1984) and 137 of the women who were randomised to receive no additional treatment have been used as a control group, their prognostic features having already been matched with the treated patients in the trial. Figure 7 shows the significant survival benefit of CMF alone for patients with both ER+ and ER- tumours; although the benefit for those with ER+ tumours is less than that seen with Tam, the benefit for those with poor prognostic ER- tumours is obvious (69% survival in ER- treated patients vs 23% survival in ER controls after 5 years and 52% vs 18% respectively after 10 years ($\chi^2 = 54.27, P < 0.0001$).

Adjuvant CMF + Tam compared with CMF alone

In order to assess the additional benefit of Tam, the survival of the group of patients given combination
Figure 1 Overall survival according to oestrogen receptor status of patients who received no adjuvant treatment.

Figure 2 Survival curve for 57 women with grade I tumours less than 2 cm and node-negative breast cancer compared with the curve of the mean expected survival for 57 women from the general population.
Figure 3 Overall survival according to oestrogen receptor status of patients who received any adjuvant treatment.

Figure 4 Overall survival according to oestrogen receptor status of patients who received adjuvant tamoxifen.
Figure 5 Overall survival according to oestrogen receptor status of patients who received adjuvant chemotherapy.

Figure 6 Overall survival according to oestrogen receptor status of patients who received adjuvant chemotherapy and tamoxifen.
adjuvant therapy was compared with the survival of those given CMF alone. \( \chi^2 = 11.41, P = 0.010; \) Fig. 8). The patients with ER− tumours had a similar survival irrespective of the type of adjuvant treatment they received (5-year survival 60%, 10-year survival 50%). For the patients with ER+ tumours there was little difference between the two treatments for the first 5 years (CMF+Tam 84%, CMF alone 78%). This difference slightly increased after 10 years (CMF+Tam 70%, CMF alone 58%), suggesting that with longer follow-up patients with ER+ tumours may be found to benefit from the addition of Tam to CMF.

Survival, ER status and histological grade in relation to adjuvant treatment

As might be expected, the majority of grade I tumours were ER+ (230/260, 88%) and patients with these tumours did well, irrespective of treatment, with only three deaths occurring within 10 years of treatment. The effect of treatment did not appear to be modified by the nature of grade II tumours, patients with ER+ tumours receiving adjuvant therapy faring better than those with ER− tumours, (10-year survival: ER+ 65%, ER− 50%). An interesting non-significant trend was observed in the women with grade III tumours; initially those with ER+ tumours had a better survival than those whose tumours were ER−, but after 8 years the survival curves crossed over and with longer follow-up the ER+ patients appeared to do worse (5-year survival ER+ 76%, ER− 68%; 15-year survival ER+ 50%, ER− 60%; Fig. 9). This trend was not modified by treatment, as the curves were similar irrespective of whether or not adjuvant therapy was given. The numbers were too small for any further analysis.

Discussion

This analysis of the relationship between ER status, survival and different adjuvant treatment regimes has enabled us to draw some conclusions regarding the various treatment groups but has highlighted the problems of assessing the significance of prognostic markers in patients receiving these different treatments and of comparing the benefits of the different regimes. Direct comparison between treatment regimes is not possible, largely due to patients with different prognostic features being selected to receive different treatments. In the present study this is confounded by the lack of suitably matched control patients, particularly for the Tam-treated patients. With the increased use of adjuvant therapy in the management of patients with breast cancer this problem will become universal.
Figure 8 Overall survival according to oestrogen receptor status of patients who received adjuvant chemotherapy and tamoxifen (CMF + Tamoxifen) compared with patients who received adjuvant chemotherapy alone.

Figure 9 Overall survival according to oestrogen receptor status of all patients (both treated and untreated) with grade III infiltrating ductal carcinomas.
The most significant effect of ER status is seen in patients in the study treated with adjuvant Tam, where those with ER+ tumours benefited considerably more than those with ER− tumours. This is in agreement with the most recent overview (Early Breast Cancer Trialists’ Group 1998a), which clearly demonstrates a greater benefit to the ER+ women. In contrast to the predominantly postmenopausal (92%) Tam-treated patients, those who received chemotherapy alone or in combination with Tam were younger (72% premenopausal) and, more importantly, had a much higher percentage of node positivity (>80% vs 55%) and incidence of grade III infiltrating carcinomas (>50% vs 30%). For those given chemotherapy alone an advantage for patients with ER+ tumours is less marked and the difference in survival is only marginally significant. This could well be due to the considerable benefit of chemotherapy on ER− patients.

This is in agreement with our previous study, which included many of the patients reviewed here, where the greatest benefit of adjuvant CMF was also seen in women with ER− tumours (Miles et al. 1999). The patients who received combined chemo/endocrine treatment showed similar behaviour to the chemotherapy alone group up to 5 years. Follow-up is insufficient to comment reliably on 10-year survival but, as shown in Fig. 6, there is a suggestion that with longer follow-up the benefit to patients with ER+ tumours is more pronounced.

From this study, it is clear that ER status was not an informative prognostic marker in our patients treated by surgery alone. The results may have been confounded by the fact that apart from the control patients from trials, the majority of those with poor prognostic features have been selected out to receive adjuvant therapy. We believe it is important to look at specific features of tumours which may predict response to different treatment regimens, rather than looking only at response in the patient population overall. There may well be an association between the marker under investigation and already established histological features of prognostic significance. For example, it is customary to examine outcome in relation to nodal status, but analyses within grade may also be informative.

Recent evidence (Roylance et al. 1999) indicates that grade actually helps to define tumours with different biologies. The suggestion from the present study that ER+ and ER− tumours of high histological grade may behave differently warrants further examination. Up to 8 years after initial treatment, patients with ER+ tumours fared slightly better but after this the survival curve for women with ER− tumours appeared to level off, whereas that for ER+ tumours continued to decline. It has also been suggested that ER+ and ER− cells arise from different portions of the developing acinus (Soler et al. 1999). Thus, both grade and ER status may help to determine tumours that are quite distinct from one another and should be so investigated (Barnes & Hanby 2001).

In agreement with other laboratories (Zafrani et al. 2000) we have noted in recent years that the percentage of women with ER positive tumours has increased. Our positive rate has risen from between 65% and 70% to 75% or more. Using immunohistochemical analysis of tissue sections it is possible to measure ER status on even the smallest carcinomas and to quantify more accurately the status of sparsely cellular lesions such as infiltrating lobular carcinomas (Barnes et al. 1996). Furthermore, with the introduction of mammographic screening programmes there is increased detection of small and well-differentiated carcinomas including those of special type. These two factors undoubtedly account for the increased percentage of patients with ER+ tumours now being detected in clinical practice (Blamey et al. 2000).

This hypothesis is supported by our findings in 560 patients treated in the Unit during the same period as those in the study, in whom ER status was not analysed, mainly due to the lack of material for the cytosol assay. These patients had tumours with more favourable characteristics than those in the study population. In particular, more of the tumours were small (<1 cm), fewer were large (>2.5 cm) and fewer patients were node positive. There was also a greater percentage of tumours of special type. The majority of these tumours would almost certainly have been ER+. Log-rank analyses on these 560 patients confirmed their excellent prognosis (data not shown). This is consistent with our finding in patients with known ER status who received no adjuvant treatment. Those with node-negative, small, low grade tumours had a survival similar to that of the averaged survival taken from the table of expected death rates for women matched for age from the general population. Similar results have been reported previously from this Unit on women with tumours having favourable prognostic features (O’Reilly et al. 1990). In view of these findings it is questionable as to whether adjuvant treatment is necessary in such patients.

Finally, the currently increasing use of adjuvant therapy may invalidate many studies of both prognostic and predictive markers since selection criteria for specific treatment result in both markers and therapy influencing duration of survival. This is an important consideration, which must be remembered when analysing the relevance of new markers, particularly in small studies. That is not to say that markers are no longer important, but care is needed when assessing their relevance and that of the proteins they identify. The future of prognostic markers probably lies in guiding treatment rather than providing a
definitive prediction of survival. ER status, which was initially used in the selection of therapy for patients with advanced disease, is now clearly established as a major predictive factor in the treatment of primary disease and should be used as a selection criterion in this setting.

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