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Two years of adjuvant tamoxifen in premenopausal patients with breast cancer: a randomised, controlled trial with long-term follow-up

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Abstract

Adjuvant tamoxifen treatment increases recurrence-free survival (RFS) and overall survival (OS) in early breast cancer, although in premenopausal patients the number of studies comparing tamoxifen *vs* no treatment are limited. We report herein the effect on RFS of adjuvant tamoxifen treatment in a multicentre trial of premenopausal patients with stage II breast cancer patients randomised between 1986 and 1991 to 2 years of tamoxifen treatment ($n = 276$) or no treatment ($n = 288$). The receptor status of the tumour was known for 541 (96%) of the patients included. Tamoxifen treatment significantly increased RFS in patients with hormone receptor-positive (oestrogen receptor-positive (ER+) and/or progesterone receptor-positive (PR+)) tumours (Relative Risk (RR) 0.65; 95% Confidence Interval (CI): 0.48–0.89, $P = 0.006$), and the beneficial effect of tamoxifen was extended to patients with indicators of poor prognosis, such as young age and nodal-positivity. PR status was a significant predictor of response to tamoxifen in multivariate models with testing of interactions of hormone receptor status and adjuvant therapy.
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Keywords: Early breast cancer; Premenopausal; Adjuvant tamoxifen; Predictive markers; Hormone receptor content; Long-term follow-up

1. Introduction

Adjuvant tamoxifen treatment increases recurrence-free survival (RFS) and overall survival (OS) in breast cancer patients with hormone receptor-positive tumours irrespective of their nodal status, menopausal status or age [1]. The tamoxifen effect is best established in postmenopausal patients, where data from our group and others supports the beneficial effect of prolonged tamoxifen treatment (5 years) compared with 2 years [1–5]. In premenopausal patients, 5 years of adjuvant tamoxifen reduces the risk of recurrence and death, irrespective of whether chemotherapy has been given [1]. An overview analysis suggests that a shorter duration (1 or 2

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years of tamoxifen) of treatment in premenopausal patients has less clinical effect and no effect of tamoxifen treatment has been reported from individual studies [1,6]. Only a few trials investigated tamoxifen as monotherapy in this group of patients, while several studies compared chemotherapy and/or oophorectomy *vs* tamoxifen [7–9].

Oestrogen receptor content (ER) is the best recognised predictive marker for adjuvant tamoxifen treatment [1,10]. Progesterone receptor content (PR) has been proposed to add information and serve as an alternative predictor [11–13]. Increased proliferation in tumour cells may further predict the patient's response to adjuvant tamoxifen, which is of interest since a high S-phase rate correlates with an increased risk of relapse and death [14]. The response to tamoxifen in relation to the patient's histological grade (Nottingham measure) has been explored in retrospective materials, and a positive effect of tamoxifen in hormone-responsive disease has been reported for tumours of all histological grades [15]. New markers to predict response to tamoxifen are continuously being evaluated retrospectively, and HER2 is the most widely studied [16–19]. HER2 positivity indicates response to trastuzumab and helps to explain biologically resistance to tamoxifen in ER-positive disease.

This is the first report from a Swedish randomised study of 2 years of adjuvant tamoxifen *vs* no treatment including only premenopausal patients with stage II invasive breast cancer. The main purpose of the trial was to investigate the effect of tamoxifen on RFS in relation to the patient's ER and PR status. Data regarding OS are also presented.

2. Patients and methods

2.1. Study design

The aim of the study was to compare the effect of tamoxifen treatment *vs* no treatment (control) in relation to RFS (primary outcome) and OS (secondary outcome) in premenopausal patients. RFS included local, regional, distant recurrences and breast cancer-specific death, but not contralateral breast cancer, as the primary event. Premenopausal patients or patients under 50 years with stage II (pT2 N0 M0, pT1 N1 M0 and pT2, N1 M0) invasive breast cancer were included. Stratification for tumour size or nodal status was not included in the protocol. Patients were considered premenopausal until one year after the last menstruation and were included independent of their hormone receptor status.The study was approved by the ethical committees at Lund and Linköping Universities.

From 1986 to 1991, 564 patients from two study centres in Sweden were enrolled in the trial and randomised to receive adjuvant tamoxifen ($n = 276$) or no treatment (control) ($n = 288$). Study centre 1 (South–East region) included 137 patients and study centre 2 (South region) 427 patients. The trial was planned to include at least 500 patients in a two-armed study aiming at a 15% difference in outcome between the arms, with 90% power and an alpha level of 5%. Randomisation was performed by the Regional Oncological Centres and oral informed consent was registered for all patients.

All patients received radical surgery in the form of a modified radical mastectomy or breast-conserving surgery with axillary lymph node dissection (levels one and two). After breast-conserving surgery, radiotherapy (50 Gy) was given to the breast, and in patients with axillary lymph node metastases, locoregional radiotherapy was delivered. Study centre 1 used a daily dosage of 40 mg tamoxifen, whereas study centre 2 used a daily dosage of tamoxifen 20 mg. In postmenopausal patients, the results of tamoxifen treatment 20 mg or 40 mg daily have been reported to be similar [2]. Adjuvant polychemotherapy with cyclophosphamide, methotrexate and fluoro-uracil (CMF) or goserelin was administered in nine patients (less than two percent).

The patients were followed upto 5 years with an annual mammogram and physical examination and then within the national programme by means of a screening mammogram every 18 months. The follow-up schedule was identical in both arms of the study. The median duration of follow-up for patients without a breast cancer event was 13.9 years (95% Confidence Interval (CI): 13.6–14.3), and median follow-up time was the same in the two treatment arms. The trial profile is given in Fig. 1. Patients' records were re-evaluated from 2002 to 2003 to ensure that the patients were premenopausal at the time of randomisation and to extend the follow-up period. Causes of death were obtained from Statistics Sweden.

Clinical and tumour characteristics in the two study groups are presented in Table 1. The characteristics of the treatment groups were similar, with the exception of a higher proportion of larger tumours in the patients who received tamoxifen therapy.

2.2. Biological characteristics

2.2.1. Oestrogen and progesterone receptor content

Hormone receptor analyses were performed in 453 patients at the time of primary surgery. ER content was measured with isoelectric focusing (IF) in polyacrylamide gels or enzyme immuno-assay (EIA), and PR content with EIA, IF, or a dextran-coated charcoal method (DCC) with Scatchard analysis [20–22]; EIA was performed according to the kit instructions (Abbott Laboratories, Diagnostic Division, Chicago, Illinois, USA). Comparisons between the different techniques and different reference parameters (protein and DNA) have yielded satisfactory concordance [3,14]. Samples

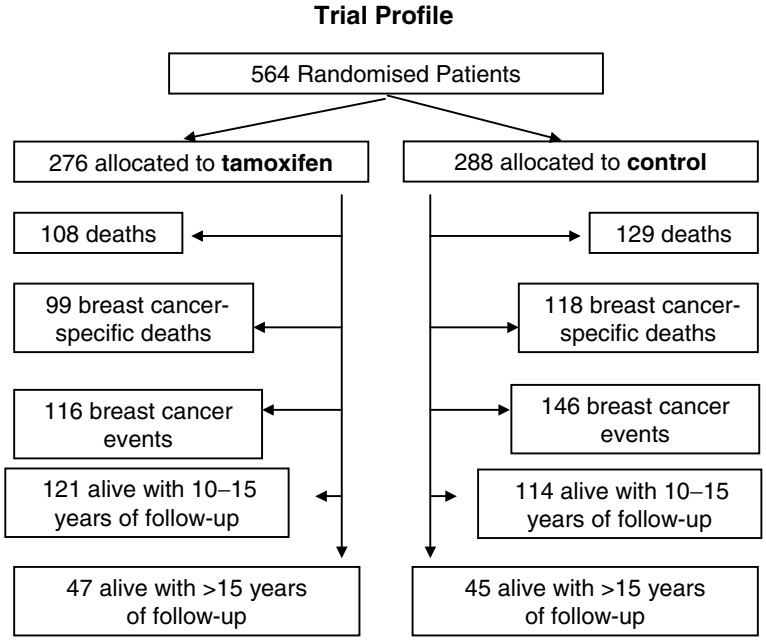

Fig. 1. Trial profile.

Table 1
Clinical data and tumour characteristics according to treatment arm

Variable	Control arm (n = 288)	Tamoxifen-treated arm (n = 276)	P-value (χ^2-test)
Age (years)			
Median (range)	45 (26–57)	45 (25–57)	
<40	61	52	0.72
40–49	184	178	
50+	43	46	
CMF treatment	4	4	
Goserelin treatment	0	1	
Lymph node status			
0	77	83	0.50
1–3	140	135	
4+	70	57	
Unknown	1	1	
Tumour size (mm)			
Median (range)	22 (2–50)	25 (5–75)	
0–20 mm	122	85	0.005
21+ mm	166	190	
Unknown	0	1	
ER and PR status			
ER− and PR−	76	82	0.88
ER− and PR+	28	20	
ER+ and PR−	19	14	
ER+ and PR+	156	143	
ER+ and/or PR+	203	182	0.33
Unknown	9	17	
NHG			
1	31	27	0.71
2	118	104	
3	116	118	
Unknown	23	27	

Abbreviations: CMF = cyclophosphamide, methotrexate and fluorouracil, ER = oestrogen receptor, PR = progesterone receptor, NHG = Nottingham histological grade.

with receptor content $\geqslant$ 100 (IF) or $\geqslant$ 250 (EIA) fmol/mg DNA (Centre 1) and $\geqslant$ 10 (IF and DCC) or $\geqslant$ 25 (EIA) fmol/mg protein (Centre 2) were classified as ER- or PR-positive, and samples with values below these levels as ER- or PR-negative. Reproducibility studies between these two centres have demonstrated highly concordant results (ER status: 98.9% (n = 90); PR status: 97.8% (n = 90)) [3,23].

ER and PR were additionally determined by means of immunohistochemistry in 88 patients using a tissue microarray. For this purpose, the Ventana Benchmark system (Ventana Medical Systems Inc., AZ, USA) with prediluted antibodies (Anti-OR Clone 6F11 and Anti-PgR Clone 16) was used. In line with the clinically established cut-off used for hormone receptor assessment, tumours with more than 10% positively staining nuclei were considered positive. The tissue microarrays were constructed using a manual arrayer (Beecher Inc, WI,

USA) for ER and a robotised arrayer (ATA-27, Beecher Inc, WI, USA) for PR. In brief, areas representative of invasive cancer were marked on the haematoxylin-stained slides, and two 0.6 mm tissue cores were taken from each case and mounted in a recipient block, with a total amount of approximately 200 cores/block.

Comparison between the two techniques of determining hormone receptor status in 371 patients resulted in 86% concordance for ER status (κ-value = 0.70) and 88% concordance for PR status (κ-value = 0.73). A combination of the methods could therefore be used, and hormone receptor status was determined by means of cytosolic methods in 453 patients and by means of immunohistochemistry in 88 patients, a total of 541 patients (96%).

2.2.2. Nottingham histological grade

Paraffin blocks from the primary tumours were available in 500 patients and Nottingham histological grade (NHG) was re-evaluated in 491 tumours. Grading was performed following a written protocol according to the method described by Elston and colleagues [24,25]. All tumour slides available in each case were assessed in order to evaluate the percentage of tubule formations. Considering only the invasive cancer component, nuclear atypia and mitotic frequency were evaluated in the most proliferative, atypical and non-necrotic areas. Mitoses were counted at a magnification of 400 x and the cut-off points of mitoses were adjusted to field diameter. Each evaluated variable (tubule formation, nuclear atypia, maximum mitotic count) scored 1, 2 or 3, and the sum of the scores was used to ascribe the NHG: 3–5 points = NHG 1, 6–7 points = NHG 2 and 8–9 points = NHG 3. The distribution of NHG in relation to treatment arm is given in Table 1. NHG 1 and 2 constituted one group in survival analyses owing to the relatively small number of patients with NHG 1.

2.2.3. Statistics

Differences in distributions between the two arms, for clinical data and tumour characteristics, were evaluated by means of the χ^2 test. All analyses were performed using the intention-to-treat rule. RFS and OS were estimated according to the Kaplan–Meier method and by the log-rank test. Cox univariate regression analyses were used to compare survival in different subgroups. The Cox multivariate proportional hazards model was fitted to explore the effect on RFS of tumour size, lymph node status, ER and PR content, age and NHG in the untreated arm. Three different Cox models were fitted for treatment–interaction analyses including hormone receptor status, tamoxifen treatment and an interaction variable for tamoxifen treatment and hormone receptor status (tamoxifen (+/−) x hormone receptor status (+/−)). All calculations were made with Statistical Package for the Special Sciences (SPSS) 11.0 Inc., IL, USA.

3. Results

3.1. All patients

There were 262 primary breast cancer events among the included patients – 116 in the tamoxifen group and

Table 2
Recurrence-free survival with Cox univariate and multivariate analyses for 288 untreated patients

Covariate category	Univariate		Multivariate	
	RR (95% CI)	*P* value	RR (95% CI)	*P* value
Age				
Continuous variable	0.97 (0.94–0.99)	0.012	0.97 (0.94–0.995)	0.024
Lymph node status				
N0	1.00		1.00	
N+	1.94 (1.26–2.96)	0.002	2.51 (1.56–4.04)	0.0002
Tumour size				
⩽20 mm	1.00		1.00	
21+ mm	1.28 (0.92–1.80)	0.146	1.36 (0.93–1.99)	0.111
ER+ and/or PR+				
Negative	1.00		1.00	
Positive	0.85 (0.59–1.24)	0.41	1.17 (0.76–1.81)	0.482
NHG				
1–2	1.00		1.00	
3	1.82 (1.30–2.54)	0.0005	1.85 (1.23–2.78)	0.003

Abbreviations: ER = oestrogen receptor, PR = progesterone receptor, NHG = Nottingham histological grade, RR = relative risk, 95% CI = 95% confidence interval.

146 in the control group. Two years of tamoxifen treatment significantly increased the RFS, (Relative Risk (RR) 0.77; 95% Confidence Interval (CI): 0.60–0.98, $P = 0.033$), and the 10-year RFS was 60.3% in the tamoxifen-treated arm and 52.7% in the control arm. Death from any cause was recorded in 237 patients (108 in the tamoxifen-treated arm and 129 in the control arm), but tamoxifen treatment had no statistically significant effect on OS (RR 0.86; 95% CI: 0.66–1.10, $P = 0.23$). Statistically, there was no difference in terms of the response to tamoxifen between the two study centres (log-rank test, $P = 0.97$).

In untreated patients, three independent prognostic covariates were identified by Cox multivariate analysis for RFS: NHG, node-status and age (Table 2). Node-positivity and NHG 3 defined patients at high risk of recurrence, whereas increasing age identified patients at low risk of recurrence. Hormone receptor status and tumour size had no significant effect on RFS in this study.

3.2. Tamoxifen response in patients with hormone receptor-positive disease

The effect of tamoxifen treatment was explored in relation to hormone receptor status, and RFS was significantly increased in 385 patients with hormone receptor-positive disease (ER+ and/or PR+), (RR 0.65; 95% CI: 0.48–0.89, $P = 0.006$), whereas in 158 patients with ER− and PR− tumours no such effect was recorded (RR 1.03; 95% CI: 0.66–1.60, $P = 0.89$) (Fig. 2(a) and (b)). For hormone receptor-positive patients, the 10-year

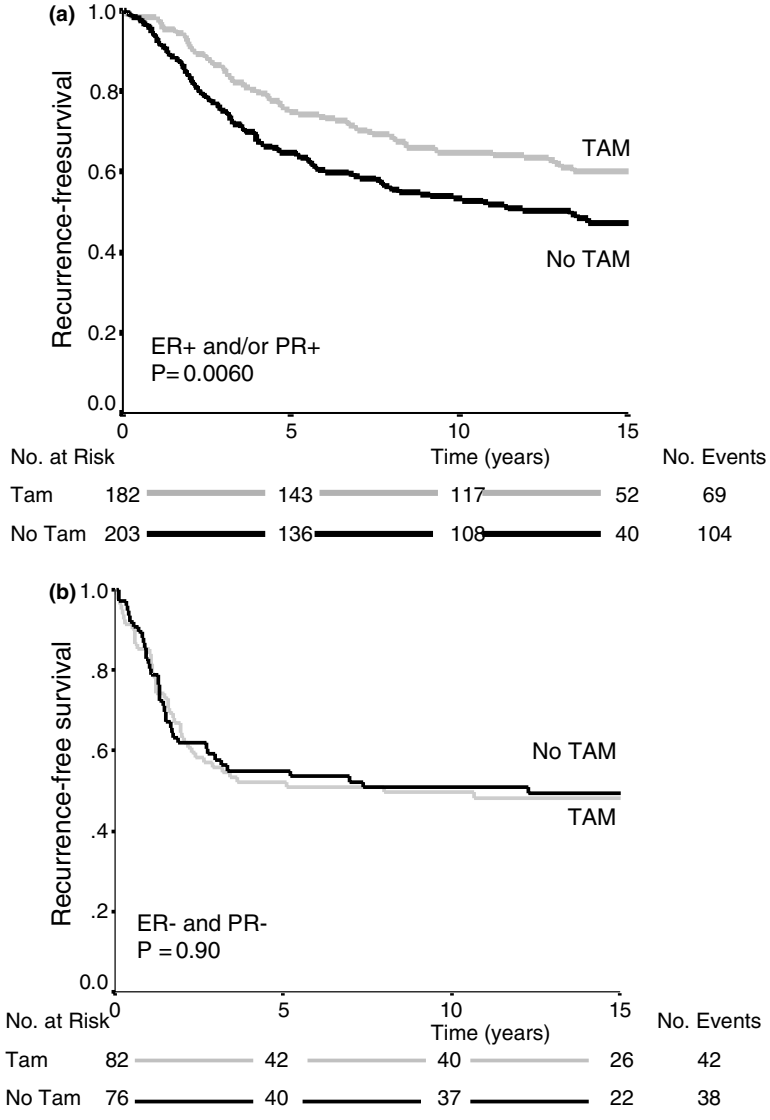

Fig. 2. Kaplan–Meier estimate for patients according to treatment arm: (a) of recurrence-free survival (RFS) for oestrogen receptor-positive (ER+) and/or progesterone receptor-positive (PR+) ($n = 385$), (b) of RFS for oestrogen receptor-negative (ER−) and progesterone receptor-negative (PR−) ($n = 156$). The log-rank test was used to calculated the P-values.

RFS was 64.8% in the tamoxifen-treated arm and 53.5% in the control arm. The tamoxifen effect in terms of OS in hormone receptor-positive patients and hormone receptor-negative patients is presented in Fig. 3(a) and (b). 10-years in OS in the TAM group was 69.8% and in the non-treated group 65.0% (giving an absolute risk reduction at 10 years of 4.8%).

Table 3 shows the tamoxifen response in relation to hormone receptor status for RFS and OS. A positive effect on RFS was observed in all subgroups of hormone receptor-positive patients (ER+, PR+, ER+ and/or PR+ and ER+ and PR+). Regarding OS, no significant effect was noted in connection with tamoxifen therapy, but there was a marked trend in favour of tamoxifen treatment for patients with PR+ disease. The interaction between tamoxifen treatment and hormone receptor status on RFS and OS was further explored by three Cox models, including covariates for three groups of hormone receptor status (ER status, PR status, and a combination of ER and PR status), a treatment variable and an interaction variable. A statistically significant interaction between PR and tamoxifen was observed in an analysis of RFS. The estimated tamoxifen effect on RFS in PR+ and PR− was RR = 0.61 and RR = 1.16, respectively ($P = 0.015$), but the same analysis for OS was not statistically significant ($P = 0.19$). The interaction between ER status and the combination of ER status and PR status and the benefit from tamoxifen did not reach statistical significance (ER: $P = 0.19$ for RFS and $P = 0.52$ for OS, ER+ and/or PR+: $P = 0.11$ for RFS and $P = 0.53$ for OS, respectively).

The tamoxifen effect in patients with hormone receptor-positive disease was further explored in strata of independent predictors for RFS identified in untreated patients; age, node status and NHG (Table 4). The positive effect of tamoxifen treatment was extended to

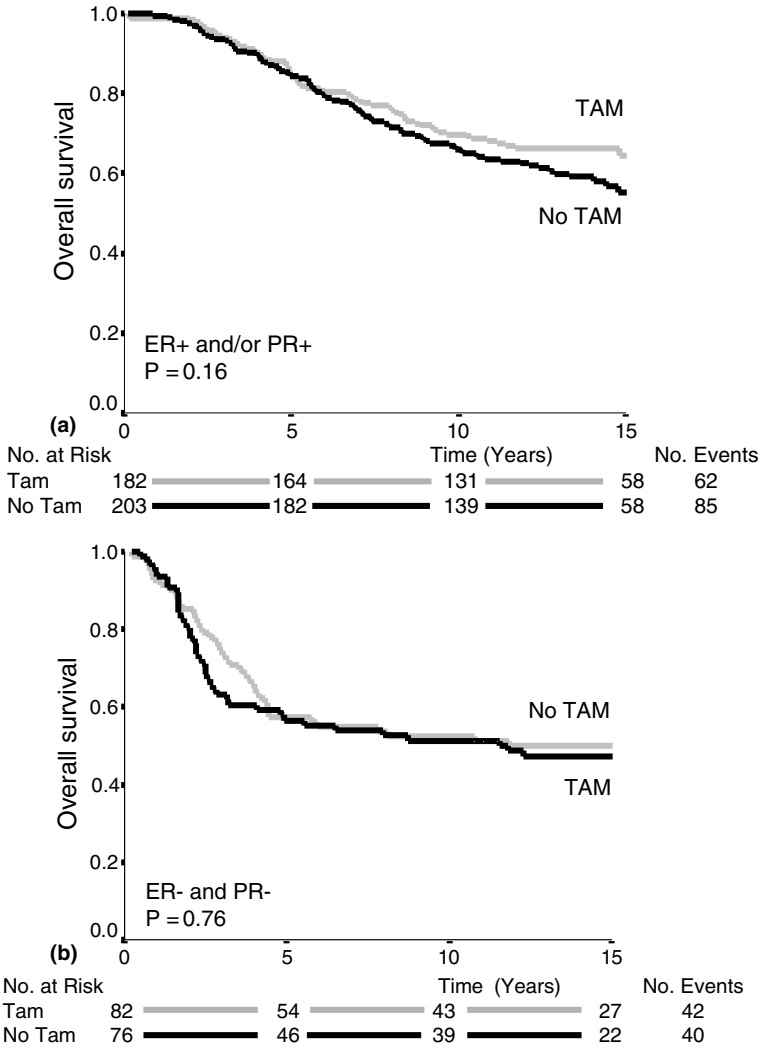

Fig. 3. Kaplan–Meier estimate for patients according to treatment arm (a) of overall survival (OS) for ER+ and/or PR+ ($n = 385$) and (b) of OS for ER− and PR− ($n = 156$). The log-rank test was used to calculated the P-values.

Table 3
Cox univariate analyses for recurrence-free survival and overall survival. Relative risk for control versus tamoxifen treatment in relation to hormone receptor content

Covariate category	Recurrence-free survival		Overall survival	
	RR (95% CI)	*P*-value	RR (95% CI)	*P*-value
ER−				
Control	1.00		1.00	
Tamoxifen	0.93 (0.64–1.35)	0.71	0.93 (0.64–1.36)	0.71
ER +				
Control	1.00		1.00	
Tamoxifen	0.65 (0.47–0.91)	0.01	0.79 (0.55–1.13)	0.19
PR−				
Control	1.00		1.00	
Tamoxifen	1.16 (0.77–1.75)	0.47	1.05 (0.70–1.57)	0.82
PR +				
Control	1.00		1.0	
Tamoxifen	0.61 (0.44–0.83)	<0.01	0.75 (0.53–1.05)	0.09
ER− and PR−				
Control	1.00		1.00	
Tamoxifen	1.03 (0.66–1.60)	0.90	0.94 (0.61–1.44)	0.76
ER+ and/or PR+				
Control	1.00		1.00	
Tamoxifen	0.65 (0.48–0.89)	<0.01	0.79 (0.57–1.10)	0.16
ER+ and PR+				
Control	1.00		1.00	
Tamoxifen	0.59 (0.41–0.85)	<0.01	0.73 (0.50–1.07)	0.11

Abbreviations: ER = oestrogen receptor status, PR = progesterone receptor status, RR = relative risk, 95% CI = 95% confidence interval.

Table 4
Recurrence-free survival with Cox univariate analyses for ER+ and/or PR+ patients. Relative risk for control versus tamoxifen treatment groups

Covariate category	*N*	Univariate	
		RR (95% CI)	*P*-value
Age (years)			
<40			
Control	37	1.00	
Tamoxifen	25	0.49 (0.24–0.99)	0.046
40+			
Control	166	1.00	
Tamoxifen	156	0.72 (0.51–1.01)	0.057
Lymph node status			
N0			
Control	48	1.00	
Tamoxifen	51	0.67 (0.34–1.30)	0.233
N+			
Control	154	1.00	
Tamoxifen	130	0.67 (0.48–0.94)	0.021
NHG			
1–2			
Control	135	1.00	
Tamoxifen	116	0.63 (0.42–0.93)	0.021
3			
Control	57	1.00	
Tamoxifen	54	0.62 (0.38–1.02)	0.060

Abbreviations: ER = oestrogen receptor status, PR = progesterone receptor status, NHG = Nottingham histological grade, RR = relative risk, 95% CI = 95% confidence interval.

patients with node-positive disease and patients at young age (<40 years). In the limited group of node-negative patients ($n = 99$) where there were only a few events, no statistically significant effect of tamoxifen was recorded.

4. Discussion

The present study demonstrates a positive effect for 2 years treatment of adjuvant tamoxifen as monotherapy *vs* control with regard to RFS. The tamoxifen effect was only observed for patients with hormone receptor-positive tumours (ER+ and/or PR+) and seems to have no effect in patients with ER− and PR− tumours. In hormone receptor-positive disease, the beneficial effect of 2 years of tamoxifen was extended to patients at high risk of recurrence, including young patients (<40 years) and those with positive nodal disease. Interestingly, PR positivity was an independent and significant predictor of the effect of tamoxifen on RFS. This finding is in agreement with previous reports by our group and others from non-randomised studies observing that PR status is valuable for predicting tamoxifen response in both pre- and post-menopausal patients [3,11–14].

The absolute risk reduction after 10 years of follow-up in this study for hormone receptor-positive disease following 2 years of tamoxifen treatment is 11.3% for RFS, and can indirectly be compared with the absolute risk reduction at 10 years for adjuvant polychemotherapy (CMF) in ER-positive disease in patients under 50 years (11.2% for RFS reported in EBCTG's overview analysis) [26]. In terms of survival, CMF increased OS by 6.8%, whereas the positive effect on OS of 2 years of tamoxifen treatment was 4.8%. For premenopausal patients with hormone receptor-positive disease, the effect of prolonged and combined endocrine therapy is even more pronounced [9]. Fortunately, the effects of endocrine therapy and chemotherapy tend to be independent of each other, and patients can receive the full benefit of both types of therapy [1].

The beneficial effect of 5 years of adjuvant tamoxifen in postmenopausal patients with hormone receptor-positive disease was demonstrated by the Swedish Breast Cancer Group [3] and extended to patients under 50 years by the Oxford meta-analysis of this and other studies [1]. The results from trials exploring shorter duration of tamoxifen therapy in premenopausal patients remain doubtful, owing to the limited

number of trials [1]. Adjuvant tamoxifen alone was considered ineffective for premenopausal patients before 1998, and randomised clinical trials to premenopausal patients have therefore included only few studies where the effect of tamoxifen alone can be explored [1,6,7,9]. This randomised study including only premenopausal patients allocated to 2 years of adjuvant tamoxifen treatment *vs* no treatment contributes substantially to the data regarding the efficacy of tamoxifen alone in this group of patients. The beneficial effect on RFS of 2 years of tamoxifen alone in hormone receptor-positive patients has been established, but the effect of prolonged tamoxifen treatment in terms of survival should be explored further. Particularly given that patients with PR-positive tumours in our study showed a trend in favour of tamoxifen treatment with regard to OS.

To conclude, adjuvant tamoxifen for 2 years in premenopausal patients significantly increases RFS in hormone receptor-positive breast cancer. This effect was identified in all patients with hormone receptor-positive disease. The interaction between PR status and tamoxifen treatment was statistically significant for RFS, showing that PR status may have predictive properties that are stronger than ER status in this group of patients.

Conflict of interest

None declared.

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