ORIGINAL ARTICLE

Pathology parameters and adjuvant tamoxifen response in a randomised premenopausal breast cancer trial

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Background: Subgroups of breast cancer that have an impaired response to endocrine treatment, despite hormone receptor positivity, are still poorly defined. Breast cancer can be subdivided according to standard pathological parameters including histological type, grade, and assessment of proliferation. These parameters are the net result of combinations of genetic alterations effecting tumour behaviour and could potentially reflect subtypes that respond differently to endocrine treatment.
Aims: To investigate the usefulness of these parameters as predictors of the response to tamoxifen in premenopausal women with breast cancer.
Materials/methods: Clinically established pathological parameters were assessed and related to the tamoxifen response in 500 available tumour specimens from 564 premenopausal patients with breast cancer randomised to either two years of tamoxifen or no treatment with 14 years of follow up. Proliferation was further evaluated by immunohistochemical Ki-67 expression.
Results: Oestrogen receptor positive ductal carcinomas responded as expected to tamoxifen, whereas the difference in recurrence free survival between control and tamoxifen treated patients was less apparent in the relatively few lobular carcinomas. For histological grade, there was no obvious difference in treatment response between the groups. The relation between proliferation and tamoxifen response seemed to be more complex, with a clear response in tumours with high and low proliferation, whereas tumours with intermediate proliferation defined by Ki-67 responded more poorly.
Conclusions: Clinically established pathology parameters seem to mirror the endocrine treatment response and could potentially be valuable in future treatment decisions for patients with breast cancer.

Patients with breast cancer who have hormone receptor positive tumours benefit from adjuvant tamoxifen treatment, regardless of nodal status, menopausal status, and age.[1] Both recurrence free survival (RFS) and breast cancer survival (BCS) are improved and prolonged treatment (five years) enhances the effect.[1–5] Hormone receptor positivity is fundamental for the response to tamoxifen; oestrogen receptor (ER) status is the best established predictive marker,[1 6] and progesterone receptor (PR) status seems to be an important alternative predictor.[6 7]

Nevertheless, not all patients with breast cancer who have steroid receptor positive tumours respond to antioestrogen treatment as a result of either de novo or acquired resistance. New predictive markers for tamoxifen response are continually being investigated; HER2 is one of the best studied of these markers and is also important because of the availability of targeted treatment against this receptor.[8–10] Modern genomic and proteomic screening techniques in combination with high throughput tissue analyses/tissue microarrays have facilitated the process of identifying and evaluating candidate disease genes responsible for the clinical behaviour of tumours. Nevertheless, the different phenotypes are reflected by variations in standard clinicopathological parameters, such as histological type, histological grade, and proliferative activity. These parameters are readily assessed and constitute well established prognostic factors, although their influence upon the response to endocrine treatment needs further investigation. The beneficial effect of tamoxifen has been demonstrated in tumours of all histological grades,[11] whereas there are few reports on the relation between histological type and endocrine response. In one retrospective study of 267 invasive ductal carcinomas (IDCs) and 33 invasive lobular carcinomas (ILCs), no difference in endocrine response between the two groups was seen, despite almost all ILCs being hormone receptor positive.[12] Another, more recent study, found that ILC had a worse response to neoadjuvant chemotherapy.[13]

> "Not all patients with breast cancer who have steroid receptor positive tumours respond to antioestrogen treatment as a result of either de novo or acquired resistance"

The growth kinetics of breast tumours have a clear relation with clinical outcome, and most studies using proliferation markers have shown that they have prognostic significance[14–18] and predictive value for response to adjuvant chemotherapy.[19–23] Only a few studies have investigated the usefulness of proliferation markers for predicting response to endocrine treatment and the results are somewhat conflicting, most indicating a better response in tumours with a low proliferation rate.[24–27] Therefore, the aim of our present study was to investigate the response to adjuvant tamoxifen in relation to histological type, grade, and proliferative activity—as assessed by mitotic index (MI) and Ki-67 index—in premenopausal patients included in a randomised trial.

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Abbreviations: BCS, breast cancer survival; ER, oestrogen receptor; IDC, invasive ductal carcinomas; ILC, invasive lobular carcinoma; MI, mitotic index; NHG, Nottingham histological grade; PR, progesterone receptor; RFS, recurrence free survival

Table 1 Clinicopathological characteristics in ductal and lobular carcinomas

	IDC n = 411 (%)	ILC n = 43 (%)	p Value
Randomisation			0.99
Tamoxifen	201(48.9)	21 (48.8)	
Control	210 (51.1)	22 (51.2)	
Age			0.49
<40 years	77 (18.7)	5 (11.6)	
40–49 years	271 (65.9)	30 (69.8)	
50–59 years	63 (15.3)	8 (18)	
Tumour size			0.47
0–10 mm	24 (5.8)	3 (6.9)	
11–20 mm	133 (32.4)	10 (23.2)	
21+ mm	253 (61.5)	30 (69.8)	
Not registered	1 (0.2)		
Lymph node status			0.88
0	103 (25.0)	12 (27.9)	
1–3	212 (51.6)	20 (46.5)	
4	94 (22.9)	11 (25.6)	
Not registered	2 (0.5)		
Histological grade			<0.0001
I	47 (11.4)	1 (2.3)	
II	170 (41.3)	34 (79.1)	
III	182 (44.3)	7 (16.3)	
Not evaluated	12 (2.9)	1 (2.3)	
Mitotic index			<0.0001
1	120 (29.2)	30 (69.7)	
2	144 (35.5)	8 (18.6)	
3	135 (32.8)	4 (9.3)	
Not evaluated	12 (2.9)	1 (2.3)	
Ki-67 index			0.001
0–10%	161 (39.2)	25 (58.1)	
11–25%	105 (25.5)	5 (11.6)	
>25%	93 (25.5)	2 (4.6)	
Not evaluated	52 (12.6)	11 (25.6)	
ER status			0.01
Negative	122 (29.7)	4 (9.3)	
Positive	275 (66.9)	33 (76.7)	
Not evaluated	14 (3.4)	5 (11.6)	
PR status			0.01
Negative	118 (28.7)	4 (9.3)	
Positive	263 (64.0)	33 (76.7)	
Not evaluated	30 (7.2)	6 (13.9)	

The p values were assessed by the χ^2 test.
ER, oestrogen receptor; IDC, invasive ductal carcinomas; ILC, invasive lobular carcinoma; PR, progesterone receptor.

MATERIALS AND METHODS
Patient material
Between 1986 and 1991, 564 premenopausal patients or patients under 50 years of age with stage II (pT2 N0 M0, pT1 N1 M0, and pT2 N1 M0) invasive breast cancer were enrolled in a randomised trial of adjuvant tamoxifen treatment. The patients were staged according to the International Union against Cancer TNM classification.[28] Fifty seven of 276 patients in the tamoxifen treated arm and seventy of 288 in the control arm had four or more positive lymph nodes. Patients were included irrespective of hormone receptor status. The daily dosage of tamoxifen used was either 40 mg (study center 1) or 20 mg (study center 2). Similar results for these dosages have been found in postmenopausal patients.[5 9 10] Patients were considered premenopausal until

one year after last menstruation. Patient records were reevaluated for patients older than 50 years at inclusion to ensure that they were premenopausal at the time of randomisation. All patients underwent modified radical mastectomy or breast conserving surgery with axillary lymph node dissection. In the last group, the breast was treated with radiotherapy, in addition to locoregional radiotherapy for those patients with axillary lymph node metastases. Fewer than 2% of the patients (n = 9) received adjuvant polychemotherapy.

The median follow up for patients without a breast cancer event was 13.9 years (95% confidence interval, 13.6 to 14.3) and it did not differ between the two treatment arms. Clinical and tumour characteristics were also well balanced in the two study groups. The study is described in detail elsewhere.[29] All tumours had previously been histopathologically reevaluated and graded according to Elston and Ellis[30] in 491 cases.

Table 3 Clinicopathological characteristics in relation to Ki-67 index

	Ki-67 index			
	Low	Intermediate	High	p Value*
Total number	194 (%)	118 (%)	118 (%)	
Randomisation				0.12
Tamoxifen	85 (43.8)	66 (55.9)	57 (48.3)	
Control	109 (56.2)	52 (44.1)	61(51.7)	
Age				0.38
<40 years	32 (16.5)	24 (20.3)	26 (22.0)	
40–49 years	126 (64.9)	79 (66.9)	78 (66.1)	
50–59 years	36 (18.6)	15 12.7)	14 (11.8)	
Tumour size				0.01
0–10 mm	14 (7.2)	8 (6.8)	6 (5.1)	
11–20 mm	68 (35.0)	35 (29.7)	21 (17.8)	
21+ mm	112 (57.7)	74 (62.7)	91 (77.1)	
Lymph node status				0.56
0	52 (26.8)	31	43	
1–3	98 (50.5)	57	30	
4	43 (22.2)	50	25	
Not registered	1 (0.05)			
NHG				<0.001
I	44 (22.7)	6 (5.1)	0 (0)	
II	104 (53.6)	48 (40.7)	19 (16.1)	
III	42 (21.6)	58 (49.1)	94 (79.7)	
Not evaluated	4 (2.1)	6 (5.1)	5 (4.2)	
Mitotic index				<0.001
1	99 (51.0)	27 (22.9)	7 (5.9)	
2	63 (32.5)	49 (41.5)	30 (25.4)	
3	28 (14.4)	36 (30.5)	76 (64.4)	
Not evaluated	4 (2.1)	6 (5.1)	5 (4.2)	
Oestrogen receptor				<0.001
Negative	33 (17.0)	34 (28.8)	71 (60.2)	
Positive	155 (79.9)	81 (68.6)	44 (37.3)	
Not evaluated	6 (3.1)	3 (2.5)	3 (2.5)	
Progesterone receptor				<0.001
Negative	35 (18.0)	41 (34.7)	76 (64.4)	
Positive	147 (75.8)	68 (57.6)	37 (31.3)	
Not evaluated	12 (6.2)	9 (7.6)	5 (4.2)	

The p values were assessed by the χ^2 test.
NHG, Nottingham histological grade.

Table 2 ER positivity in relation to Ki-67 index in the two study groups

	Ki-67 index				
	0–1%	2–10%	11–25%	26–50%	51–100%
Number ER positive	42	113	81	37	7
Tamoxifen v control*	0.05	0.03	0.66	0.02	0.07

*Log rank recurrence free survival p value.
ER, oestrogen receptor.

Our study was approved by the ethical committees at Linköping and Lund Universities, Sweden.

Tissue array and immunohistochemistry

Areas representative of invasive cancer were marked on the haematoxylin and eosin stained slides and two separate tissue microarrays were constructed, one using a manual (MTA-1) and the other an automated (ATA-27) arrayer (both from Beecher Inc, Sun Prairie, Wisconsin, USA). Two 0.6 mm tissue cores were taken from each donor block and mounted in a recipient block. There were approximately 200 tissue cores in each recipient block. Cores were generally taken from the peripheral part of the tumour in cases where the tumour had relatively well defined borders. For technical reasons, in more diffusely growing tumours, areas with the highest tumour cell density were primarily targeted. Sections (4 μm thick) were dried, dewaxed, rehydrated, and microwave treated for 2×5 minutes in citrate buffer (pH 6.0) before being processed in an automatic immunohistochemistry staining machine (Techmate 500; Dako, Copenhagen, Denmark) for Ki-67, using an anti-Ki-67 monoclonal antibody (1/200 dilution; M7240; Dako). For immunohistochemical evaluation of Ki-67, a scoring system based on the estimated fractions of positively staining nuclei was used as follows: 0, 0–1%; 1, 1–10%; 2, 11–25%; 3, 26–50%; and 4, 51–100%. The intensity of the nuclear staining for Ki-67 varied slightly, but was distinct in most cases.

In addition, the tissue microarrays were analysed immunohistochemically for ER and PR status using the Ventana Benchmark system (Ventana Medical Systems Inc, Tucson, Arizona, USA), with prediluted antibodies (anti-ER clone 6F11 and anti-PR clone 16). The fractions of ER and PR positive nuclei were determined and classified into four groups (0–10%, 11–50%, 51–75%, and > 75%). In line with the clinically established cutoff value used for hormone receptor assessment, tumours with more than 10% positively staining nuclei were considered positive. The MI had previously been assessed along with histological grading, by counting the number of mitoses/10 high power fields and adjusting for the optics used.[30] Tumours had then been given a score from 1 (low) to 3 (high). For this investigation, an additional reevaluation regarding histological type[31] was performed.

Statistics

Differences in distribution between clinical data and tumour characteristics were analysed by the χ^2 test. RFS, BCS, and overall survival OS were estimated according to the Kaplan–Meier method and the log rank test was used to compare survival in different strata. RFS considered local, regional, and distant recurrences and breast cancer specific death, but not contralateral breast cancer, as a primary event. Causes of death were obtained from Statistics Sweden. For BCS, patients who died of any other cause were censored at the time of death. A Cox proportional hazards model was used for the estimation of the relative risk in univariate and multivariate analyses. The interaction between tamoxifen treatment and the investigated parameters was further explored by Cox models including one of the four variables respectively, a treatment variable and an interaction variable. All statistical tests were two sided and the calculations were performed with SPSS 11.0 (SPSS Inc, Chicago, Illinois, USA).

RESULTS
Tumour type and hormone receptor status

Paraffin wax blocks were available from 500 patients included in the randomised treatment trial and assessment of histological type was possible in 493 cases. IDCs formed the largest group (83%), followed by ILCs (8.7%). There was
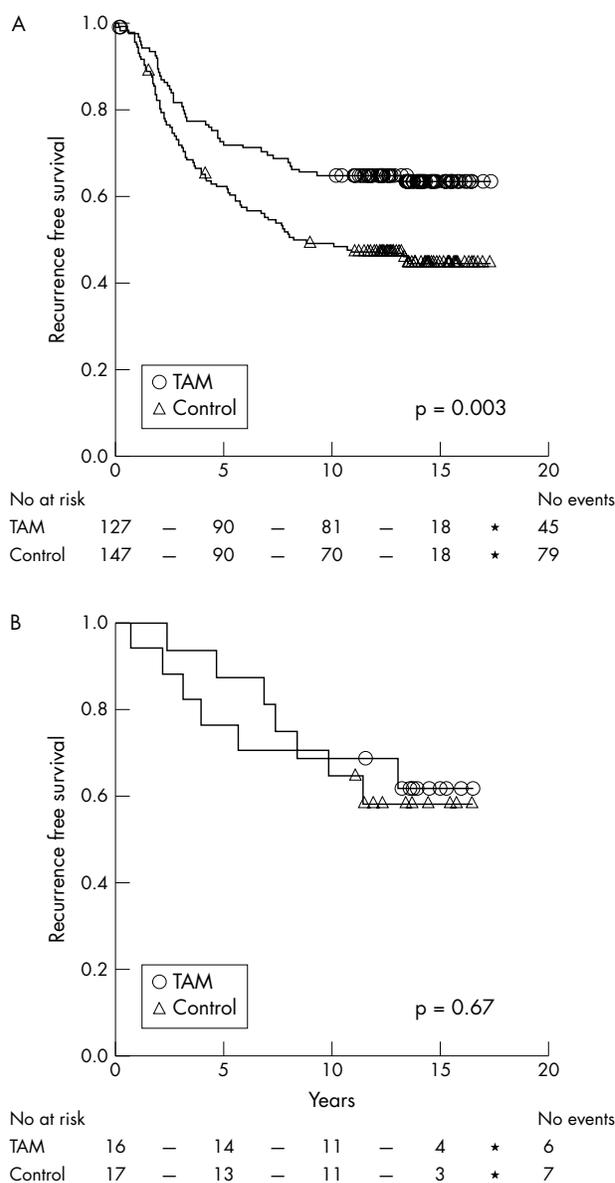

Figure 1 Kaplan–Meier estimates of recurrence free survival according to treatment arm for oestrogen receptor positive cases of (A) invasive ductal carcinomas (n = 274) and (B) invasive lobular carcinoma (n = 33). TAM, tamoxifen treated.

no significant difference in the histological type distribution between patients randomised to tamoxifen treatment or no treatment. Table 1 summarises the clinicopathological characteristics of the histological subgroups of IDC and ILC. In general, a larger proportion of ILCs were hormone receptor positive compared with IDC. In addition, most ILCs were strongly ER and PR positive, with > 75% of the nuclei being positive.

Proliferation markers and histological grade

Immunohistochemical Ki-67 expression could be evaluated in 430 cases (86%). Nottingham histological grade (NHG) including MI had previously been evaluated in 514 cases with the following distribution for MI: 1, 170 cases (33.1%); 2, 171 cases (33.3%); 3, 173 (33.6%); and for NHG: I, 58 (11.3%); II, 222 (43.2%); and III, 234 (45.5%). The fraction of Ki-67 positive cells correlated significantly with MI (p < 0.0001). In addition, in the 286 tumours that had previously been

Table 4 RFS and BCS by Cox univariate analysis in ER positive tumors

Category	RFS			BCS		
	RR	95% CI	p Value	RR	95% CI	p Value
IDC						
Control	1.00			1.00		
Tamoxifen	0.58	0.40 to 0.83	0.003	0.71	0.48 to 1.07	0.10
ILC						
Control	1.00			1.00		
Tamoxifen	0.79	0.26 to 2.36	0.67	0.91	0.28 to 2.99	0.88
Low Ki-67 index						
Control	1.00			1.00		
Tamoxifen	0.50	0.29 to 0.87	0.01	0.68	0.37 to 1.27	0.22
Intermediate Ki-67 index						
Control	1.00			1.00		
Tamoxifen	1.15	0.61 to 2.17	0.66	1.40	0.69 to 2.71	0.37
High Ki-67 index						
Control	1.00			1.00		
Tamoxifen	0.29	0.11 to 0.74	0.006	0.38	0.15 to 0.97	0.04
MI of 1						
Control	1.00			1.00		
Tamoxifen	0.63	0.34 to 1.16	0.14	0.57	0.28 to 1.12	0.13
MI of 2						
Control	1.00			1.00		
Tamoxifen	0.70	0.41 to 1.19	0.18	1.01	0.57 to 1.80	0.96
MI of 3						
Control	1.00			1.00		
Tamoxifen	0.50	0.26 to 0.99	0.04	0.62	0.31 to 1.22	0.16
Grade I tumours						
Control	1.00			1.00		
Tamoxifen	0.58	0.18 to 1.89	0.37	0.23	0.03 to 1.94	0.18
Grade II tumours						
Control	1.00			1.00		
Tamoxifen	0.63	0.39 to 1.01	0.06	0.82	0.49 to 1.40	0.47
Grade III tumours						
Control	1.00			1.00		
Tamoxifen	0.50	0.29 to 0.87	0.01	0.59	0.34 to 1.04	0.07

BCS, breast cancer survival; CI, confidence interval; IDC, invasive ductal carcinomas; ILC, invasive lobular carcinoma; MI, mitotic index; RFS, recurrence free survival; RR, relative risk.

analysed by flow cytometry,[15] there was a significant correlation between Ki-67 and S phase fraction ($p < 0.0001$; data not shown). Tables 2 and 3 show the distribution of clinicopathological parameters in the different Ki-67 groups. ER status was available for 457 cases.

Survival analyses regarding tamoxifen response
For survival analyses, all ER positive ($> 10\%$) cases were included, irrespective of PR status, which is line with the current recommendation regarding endocrine treatment. As described previously,[29] patients with ER positive tumours receiving two years of tamoxifen had a significantly higher RFS ($p = 0.005$). The increase in BCS was not significant ($p = 0.13$), although there was a more favourable trend in the tamoxifen treated group. Tamoxifen treatment had no effect in receptor negative tumours.

We next delineated the tamoxifen response in different histological subgroups of breast cancer. Tamoxifen treatment was associated with a significantly increased RFS for ER positive IDC ($p = 0.003$), whereas there was no significant difference in RFS for ILC ($p = 0.67$) (fig 1). Tamoxifen treatment also improved BCS in IDC, although it did not reach significance ($p = 0.10$). The Kaplan–Meier plot for ILC shows slightly diverging curves in favour of the tamoxifen treated group early during treatment, but the lines converged later. Thus, after five years, RFS was 72% versus 62% and 87% versus 76% for treated and untreated patients with IDC and ILC, respectively. After 10 years, RFS was 65% versus 49% and 68% versus 65% in IDC and ILC, respectively, with similar proportions at 15 years. Interestingly, overall survival after 10 years, which was similar to BCS, was 76% versus 68% and 60% versus 69% for untreated and treated patients with ILC and IDC, respectively.

In Cox univariate analyses, the relative risk of disease recurrence after tamoxifen treatment was 0.79 and 0.58 for ILC and IDC, respectively, whereas BCS was 0.91 and 0.71 respectively, with a large confidence interval for ILC, because of the relatively small number of tumours (table 4).

Initially, we characterised the tamoxifen response in the five different Ki-67 proliferation groups (table 2); there was a significant response in the two lowest groups and in the two highest groups, whereas there was no difference in survival between untreated patients and tamoxifen treated patients in the intermediate group. Therefore, in the following analyses we divided the material into three groups corresponding to: 0–10% (low), 11–25% (intermediate), and $> 25\%$ (high) expression (table 3). Using this subdivision, RFS was significantly increased in the Ki-67 low ($p = 0.01$) and high ($p = 0.006$) groups, whereas tumours in the intermediate group did not show a significant ($p = 0.66$) tamoxifen response (fig 2). BCS (fig 3) was significantly improved by tamoxifen treatment in the group with high Ki-67 expression ($p = 0.04$), but not in the low ($p = 0.22$) and intermediate ($p = 0.37$) groups. In the group with intermediate Ki-67 expression, there was a tendency towards a shorter BCS after tamoxifen treatment (fig 3B).

The MI is one of three components of the NHG and we therefore characterised the tamoxifen response in low, intermediate, and high proliferative tumours defined by the mitotic count. The trend was similar to that seen for Ki-67 with regard to RFS and BCS, although it was not as pronounced (table 4). The only significant difference in RFS was seen in the group with the highest MI ($p = 0.04$). In contrast, and in line with the Ki-67 data, BCS was not improved by tamoxifen treatment in the intermediate group ($p = 0.96$), compared with a more obviously positive,
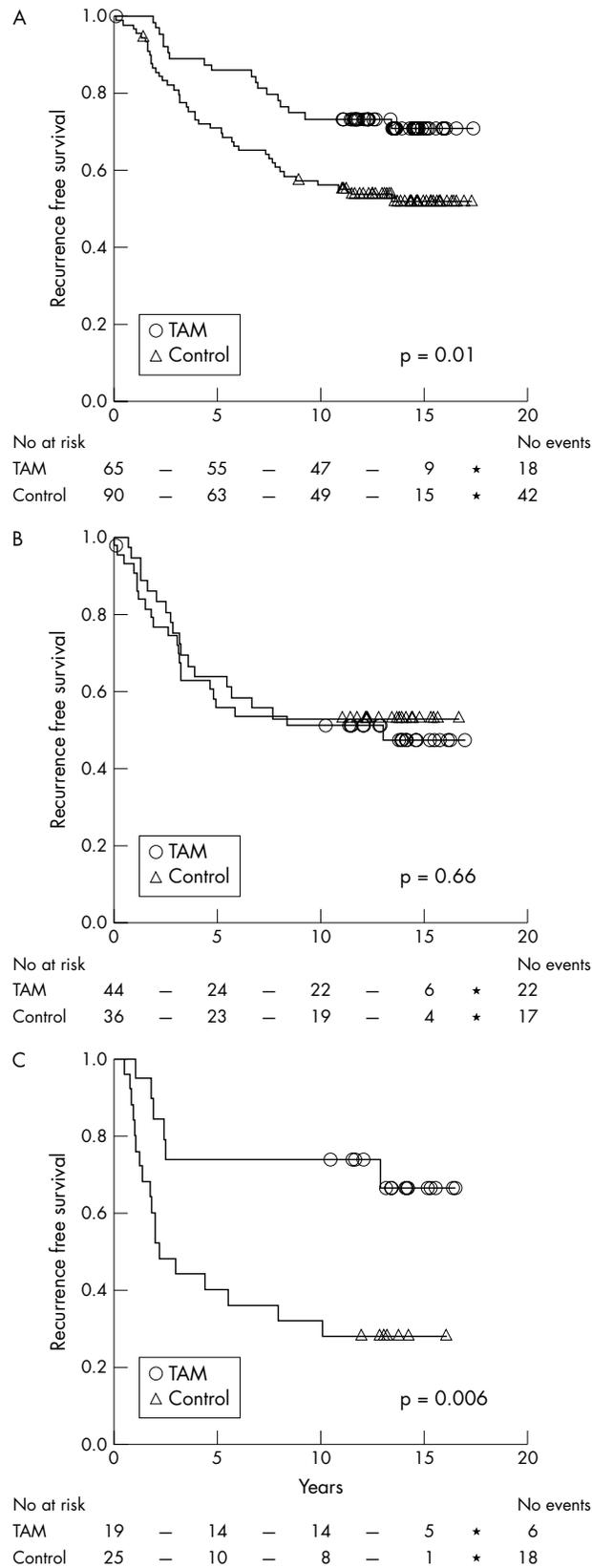
Figure 2 Kaplan–Meier estimates of recurrence free survival according to treatment arm for oestrogen receptor positive cases with (A) low, (B) intermediate, and (C) high Ki-67 expression. TAM, tamoxifen treated.
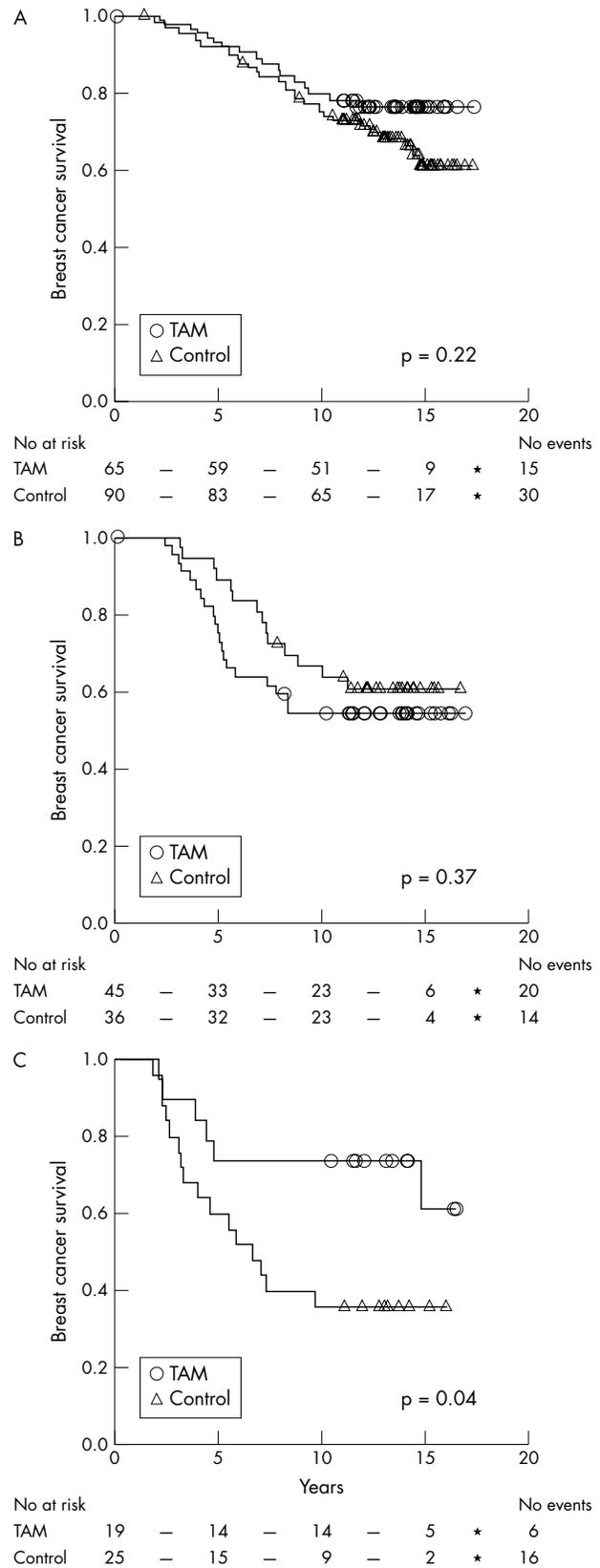
Figure 3 Kaplan–Meier estimates of breast cancer survival according to treatment arm for oestrogen receptor positive cases with (A) low, (B) intermediate, and (C) high Ki-67 expression. TAM, tamoxifen treated.

although not significant, tamoxifen effect in the low (p = 0.13) and high (p = 0.16) groups.

Regarding NHG, RFS was significantly improved for grade II and III tumours (p = 0.06 and p = 0.01, respectively), but not for the relatively few grade I tumours (p = 0.37). There was a borderline significant increase in BFS after tamoxifen treatment in grade III tumours (p = 0.07), but not in grade I and II tumours (table 4).

The interaction between tamoxifen treatment and histological type, Ki-67, grade, and MI was further explored by a Cox model including one of the four variables, a treatment variable and an interaction variable. Using this approach, a significant interaction was found between intermediate compared with low and high Ki-67 expression and tamoxifen treatment, even when adjusted for other important clinicopathological variables (table 5). None of the other variables, including histological type, showed a significant interaction with tamoxifen using this approach.

Survival analyses in patients not receiving adjuvant tamoxifen

In total, 288 patients were allocated to the control arm, which formed an untreated patient group suitable for studies of prognostic information without interference from adjuvant medication. Table 6 provides an overview of the prognostic impact of the factors investigated in our study. In the untreated group, proliferation markers in general showed a linear association with clinical outcome, with the best clinical outcome for tumours with low proliferation and worst for highly proliferating tumours.

DISCUSSION

Our study provides an ideal setting to study the endocrine response, because all patients had been randomised to tamoxifen as monotherapy, or to the control arm. Furthermore, there were no differences in the clinicopathological variables between the trial arms or different histological tumour types that could influence outcome. However, it should be noted that upon disease recurrence, tamoxifen treatment was also offered to patients initially randomised to the control group. This fact might interfere with the validity of the analyses of BCS or overall survival, but not RFS.

Histological tumour type appeared to be a predictor of tamoxifen response, with a highly significant survival benefit for RFS and almost significant benefit for BCS in IDC, in contrast to the small subset of ILCs, which did not benefit significantly from treatment. However, in multivariate interaction analyses,

no significantly different tamoxifen response could be demonstrated in IDC compared with ILC. Larger studies or meta-analyses are needed to establish such an association with acceptable statistical power.

In general, ILC shows a more diffuse, infiltrative growth pattern and is more often multifocal than IDC. However, this was not reflected by the number of local recurrences—four of 43 (9%) in ILC and 38 of 411 (9%) in IDC. Node positivity was also similar for ILC and IDC. It is clear that ILCs have different biological characteristics to IDCs, which are mirrored by specific genetic alterations in this subgroup of tumours.[32][33] The increasing incidence of ILC during the past decade[34][35] further stresses the importance of clarifying the effect of tamoxifen treatment in this breast cancer subgroup.

> "The traditional morphological examination, including evaluation of proliferation, seems to mirror the differential expression of hundreds of genes clearly linked to the clinical behaviour of tumours"

We also investigated the relation between the tamoxifen response and two proliferation markers: Ki-67 expression by immunohistochemistry and MI. The MI is routinely assessed as one of the three cornerstones of the Elston Ellis histological grading system (NHG). Interestingly, irrespective of the method used for assessing proliferative activity, the beneficial effect of tamoxifen was significant in highly proliferating tumours, both in terms of RFS and BCS, suggesting an important role for tamoxifen in this group of tumours. In contrast, those tumours with neither high nor low proliferation showed a worse response to tamoxifen. This was most apparent for Ki-67, where the high and low groups showed a significant response, whereas the intermediate group showed no response. The difference was significant for RFS and BCS, even in interaction analyses. When proliferation was defined by the MI, only highly proliferating tumours showed a significant tamoxifen response with regard to RFS, whereas the effect on BCS was similar to that seen for Ki-67, with an impaired tamoxifen response in the intermediate group. The reason why intermediately proliferating ER positive tumours have an impaired tamoxifen response is unclear. Although the choice of cutoff levels for immunohistochemical analyses, in this case Ki-67, may be disputed, our results indicate divergent behaviour for the intermediately proliferating group after tamoxifen treatment. Although this pattern may be coincidental, it must be compared with the

Table 5 Multivariate Cox proportional hazards model for Ki-67 index and treatment interaction for ER positive tumours*

Variable		RFS			BCS		
		RR	95% CI	p Value	RR	95% CI	p Value
Ki-67	Intermediate v low + high	0.85	0.50 to 1.46	0.56	0.81	0.51 to 1.69	0.93
Treatment	Tamoxifen v control	0.46	0.29 to 0.74	0.001	0.61	0.36 to 1.02	0.06
Interaction variable	Tamoxifen × Ki-67	2.63	1.19 to 5.82	0.02	2.38	1.01 to 5.63	0.05
MI	2 v 1+3	1.22	0.80 to 1.88	0.35	1.04	0.64 to 1.69	0.88
Treatment	Tamoxifen v control	0.60	0.38 to 0.94	0.02	0.66	0.40 to 1.09	0.10
Interaction variable	Tamoxifen × MI	1.18	0.59 to 2.36	0.64	1.59	0.74 to 3.39	0.23
NHG	3 v 1+2	1.86	1.17 to 2.95	0.008	2.43	1.46 to 4.06	0.001
Treatment	Tamoxifen v control	0.62	0.40 to 0.96	0.03	0.76	0.45 to 1.25	0.28
Interaction variable	Tamoxifen × NHG	0.94	0.46 to 1.92	0.87	0.96	0.44 to 2.05	0.91
Histological type	ILC v IDC	0.70	0.32 to 1.55	0.38	0.88	0.37 to 2.09	0.78
Treatment	Tamoxifen v control	0.59	0.40 to 0.85	0.005	0.75	0.50 to 1.13	0.17
Interaction variable	Tamoxifen × type	1.39	0.44 to 4.39	0.58	1.17	0.33 to 4.09	0.81

*Adjusted for age (continuous), tumour size (T1 v T2), and nodal status (N0 v N+).
BCS, breast cancer survival; CI, confidence interval; IDC, invasive ductal carcinomas; ILC, invasive lobular carcinoma; MI, mitotic index; NHG, Nottingham histological grade; RFS, recurrence free survival; RR, relative risk.

13 **Mathieu M**, Rouzier R, Llombart-Cussac A, *et al.* The poor responsiveness of infiltrating lobular breast carcinoma to neoadjuvant chemotherapy can be explained by their biological profile. *Eur J Cancer* 2004;**40**:342–51.

14 **Isola J**, Helin H, Helle M, *et al.* Evaluation of cell proliferation in breast carcinoma. Comparison of Ki-67 immunohistochemical study, DNA flow cytometric analysis and mitotic count. *Cancer* 1990;**65**:1180–4.

15 **Ferno M**, Baldetorp B, Bendahl P-O, *et al.* Recurrence-free survival in breast cancer improved by adjuvant tamoxifen—especially for progesterone receptor positive tumors with a high proliferation. *Breast Cancer Res Treat* 1995;**36**:23–34.

16 **Keshgegian A**, Cnaan A. Proliferation markers in breast carcinoma. Mitotic figure count, S-phase fraction, proliferating cell nuclear antigen, Ki-67 and MIB-1. *Am J Clin Pathol* 1995;**104**:42–9.

17 **Michels J-J**, Duigou F, Marnay J. Flow cytometry in primary breast carcinomas. Prognostic impact of proliferative activity. *Breast Cancer Res Treat* 2000;**62**:117–26.

18 **Kallioniemi O-P**, Hietanen T, Mattila J, *et al.* Aneuploid DNA content and high S-phase fraction of tumour cells are related to poor prognosis in patients with primary breast cancer. *Eur J Cancer Clin Oncol* 1987;**23**:277–82.

19 **Stal O**, Skoog L, Rutqvist L, *et al.* S-phase fraction and survival benefit from adjuvant chemotherapy or radiotherapy of breast cancer. *Br J Cancer* 1994;**70**:1258–62.

20 **Faneyte I**, Schrama J, Peterse J, *et al.* Breast cancer response to neoadjuvant chemotherapy: predictive markers and relation with outcome. *Br J Cancer* 2003;**88**:406–12.

21 **Aas T**, Geisler S, Eide G, *et al.* Predictive value of tumour cell proliferation in locally advanced breast cancer treated with neoadjuvant chemotherapy. *Eur J Cancer* 2003;**39**:438–46.

22 **Petit T**, Wilt M, Velten M, *et al.* Comparative value of tumour grade, hormonal receptors, Ki-67, HER-2 and topoisomerase II alpha status as predictive markers in breast cancer treated with neoadjuvant anthracyclin-based chemotherapy. *Eur J Cancer* 2004;**40**:205–11.

23 **Remvikos Y**, Beuzeboc P, Zajdela A, *et al.* Correlation of pretreatment proliferative activity of breast cancer with the response to cytotoxic chemotherapy. *J Natl Cancer Inst* 1989;**81**:1383–7.

24 **Silvestrini R**, Daidone M, Mastore M, *et al.* Cell kinetics as a predictive factor in node-positive breast cancer treated with adjuvant hormone therapy. *J Clin Oncol* 1993;**11**:1150–5.

25 **Daidone M**, Luisi A, Martelli E, *et al.* Biomarkers and outcome after tamoxifen treatment in node-positive breast cancers from elderly women. *Br J Cancer* 2000;**82**:270–7.

26 **Nicholson R**, Bouzubar N, Walker K, *et al.* Hormone sensitivity in breast cancer: influence of heterogeneity of oestrogen receptor expression and cell proliferation. *Eur J Cancer* 1991;**27**:908–13.

27 **Paradiso A**, Tommasi S, Mangia A, *et al.* Tumor-proliferative activity, progesterone receptor status, estrogen receptor level, and clinical outcome of estrogen receptor-positive advanced breast cancer. *Cancer Res* 1990;**50**:2958–62.

28 **International Union against Cancer (UICC)**. *TNM classification of malignant tumours.* Berlin-Heidelberg-New York: Springer-Verlag, 1987.

29 **Rydén L**, Jönsson P-E, Chebil G, *et al.* Two years of adjuvant tamoxifen in premenopausal patients with breast cancer: a randomised, controlled trial with long-term follow-up. *Eur J Cancer* 2005;**2**:256–64.

30 **Elston CW**, Ellis IO. Pathological prognostic factors in breast cancer. 1. The value of histological grade in breast cancer: experience from a large study with long-term follow-up, *Histopathology* 1991;**19**:403–10.

31 **WHO**. *International histological classification of tumours. Histologic types of breast tumours.* Geneva: World Health Organisation, 1991.

32 **Gunther K**, Merkelbach-Bruse S, Amo-Takyi B, *et al.* Differences in genetic alterations between primary lobular and ductal breast cancers detected by comparative genomic hybridization. *J Pathol* 2001;**193**:40–7.

33 **Korkola J**, DeVries S, Fridlyand J, *et al.* Differentiation of lobular versus ductal breast carcinomas by expression microarray analysis. *Cancer Res* 2003;**63**:7167–75.

34 **Li C**, Weiss N, Stanford J, *et al.* Hormone replacement therapy in relation to risk of lobular and ductal breast carcinoma in middle-aged women. *Cancer* 2000;**88**:2570–7.

35 **Verkooijen H**, Fioretta G, Vlastos G, *et al.* Important increase of invasive lobular breast cancer incidence in Geneva, Switzerland. *Int J Cancer* 2003;**104**:778–81.