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Report

Recurrence-free survival in breast cancer improved by adjuvant tamoxifen – especially for progesterone receptor positive tumors with a high proliferation

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Summary

Although the beneficial effect on breast cancer of adjuvant tamoxifen (TAM) is well established, in the series studied by our group this effect seems to have been restricted to patients with steroid receptor (especially progesterone receptor (PgR)) positive tumors. However, as some patients with PgR-positive tumors manifested recurrence despite adjuvant TAM treatment, the question arose whether some other biological factor(s) could be used to identify these non-responding cases. The level of the S-phase fraction (SPF), as measured by flow cytometry, has been shown to be a useful prognostic marker, prognosis being better in cases where the SPF is low than in those where it is high. The aim of the present study was to relate the prognosis after adjuvant TAM to SPF among patients with PgR-positive tumors.

In the PgR-positive group as a whole, the effect of TAM on prognosis was more pronounced in the high SPF group than in the low SPF group ($p = 0.005$) the respective decrease in 3 year recurrence rate was from 19 to 43% and from 17 to 9%. Multivariate analysis of the data for the TAM-treated group showed the level of PgR concentration (low positive *vs.* high positive), lymph node status, and tumor size to be independent predictive factors, but not the level of SPF (i.e. high *vs.* low). By contrast, among patients not treated with TAM, the SPF was a strong independent prognostic factor.

To sum up, SPF was a strong independent predictor of outcome only for patients receiving no systemic adjuvant therapy, but not in patients receiving adjuvant TAM. Patients with PgR-positive and high S-phase tumors derived more benefit from TAM than patients with PgR-positive and low SPF tumors.

Introduction

An overview of available trials of adjuvant tamoxifen (TAM) treatment of breast cancer comprising a total of about 30,000 patients, showed TAM treatment to be associated with significant improvement of both recurrence-free survival (RFS) and overall survival among postmenopausal breast cancer patients [1]. The beneficial effects of TAM were found to be largely restricted to patients with estrogen receptor (ER) positive tumors [2–4], whereas other have reported response to TAM to be independent of receptor status [5]. In the overview it was concluded that, although adjuvant TAM seems to have

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a beneficial effect on RFS also for patients with ER-poor tumors, this effect was much less marked than among patients with ER-positive tumors [1].

In our health care region, adjuvant TAM has been shown to improve RFS for both post- and premenopausal breast cancer patients with steroid receptor (especially progesterone receptor (PgR)) positive tumors, but not in the corresponding subgroups of patients with steroid receptor negative tumors [4, 6].

Obviously, not all breast cancer patients with steroid receptor positive tumors respond to antiestrogen therapy. PgR content has been suggested to be a more sensitive marker than ER content for predicting response to endocrine therapy, as its synthesis is dependent on estrogen stimulation, thus indicating the presence of functional ERs [7]. The importance of the concentration level for receptor-positive cases for response to endocrine therapy of both primary and metastatic breast cancer has been indicated in some studies [3–8–10]. Moreover, immunohistochemical methods have made it possible to measure receptor content at a cellular level [11]. It has been proposed that a receptor positive and homogeneous tumor (all cells containing receptors) should respond better to adjuvant TAM than a receptor-positive tumor with a heterogeneous pattern (containing both receptor positive and receptor negative cells; [11]). Moreover, in a recent study from our group, amplification of ERBB2 in PgR+ breast cancer was found to indicate an unresponsiveness to adjuvant TAM treatment [12]. The lack of response to endocrine therapy among patients with receptor-positive tumors may of course also be explained by several other mechanisms including absence of pS2 [13] and suppression of TGFβ [14].

A factor of prognostic importance in breast cancer is the S-phase fraction (SPF) in the tumor, as estimated by DNA flow cytometry [15–18]. Its usefulness in the clinical management of breast cancer is currently being evaluated in clinical trials. So far, evaluation of the SPF has largely been restricted to its use as a prognostic factor in assessing the risk of recurrence after primary treatment. The predictive value of SPF in relation to the effect of adjuvant cytotoxic treatment has been shown in some studies – tumors with a high SPF responding better to cytotoxic treatment than tumors with a low SPF [19–20]. However, to our knowledge, the importance of SPF in predicting the effect of adjuvant endocrine therapy has hitherto not been studied.

The aim of the present study was therefore to investigate the capacity of the SPF to predict the effect of adjuvant TAM for breast cancer patients with PgR+ tumors. In addition, its prognostic capacity was also investigated in a group of patients not treated with adjuvant systemic therapy.

Material and methods

Patients

Inclusion criteria

Only patients with a PgR+ tumor in which the SPF has also been estimated by flow cytometry were included (n = 647). Otherwise, the inclusion criteria were as follows: presence of tumor cells verified on imprints by a cytopathologist; availability of information on adjuvant TAM (treatment description, see below) and recurrence-free survival; and no adjuvant cytotoxic treatment. Tumor tissue for PgR measurement was obtained from 70–80% of all patients operated in the South Sweden Health Care Region, an administrative area with about 1.6×10^6 inhabitants. Residual tumor specimens obtained after steroid receptor analysis were stored frozen (– 70 °C). Part of this material has been used for flow cytometric DNA analysis, as described below. Owing to the limited amount of tissue material, information of DNA ploidy status was obtained in only 681 (54%) and S-phase fraction in 647 (52%) of cases in our total series of breast cancer patients with PgR+ tumors for whom information on adjuvant TAM treatment and recurrence-free survival was available (n = 1255). There may thus have been a selection bias, inasmuch as DNA content was not measured in smaller tumors.

The importance of SPF for the effect of adjuvant TAM treatment was first examined for all available patients (n = 647, 207 (32%) pre- and 440 (68%) post-menopausal). Of these 647 patients, 396 (61%) were treated with TAM and 251 (39%) were not. To evaluate the importance of bias favoring the selec-

tion of tumors of more advanced stage in the treated group, subgroup analyses were performed in which only patients participating in controlled randomized clinical trials were included. In addition, the importance of menopausal status was also examined. Three different groups of patients were thus investigated, of which two included only patients participating in multicenter, controlled randomized clinical trials, organized by the South Sweden Breast Cancer Group, where the effect of adjuvant treatment with TAM was investigated. The third group consisted of postmenopausal patients, irrespective of whether they participated in clinical trials or not.

Group 1: Premenopausal patients in a randomized trial (n = 130)
In a controlled multicenter clinical trial of adjuvant systemic therapy in premenopausal, stage II breast cancer patients, adjuvant TAM (20 mg daily) for two years was compared with no adjuvant systemic therapy. The patients were randomized following modified radical mastectomy. All node-positive patients received postoperative radiotherapy. The trial started in 1985 and ended in 1990.

Group 2: Postmenopausal patients in a randomized trial (n = 244)
In a controlled multicenter clinical trial of adjuvant systemic therapy in postmenopausal, stage II breast cancer, adjuvant TAM (20 mg daily) for two years was compared with the same therapy given for five years. The patients were randomized following modified radical mastectomy. All node-positive patients received postoperative radiotherapy. The trial started in 1985 and is still in process. In the present study, differences in the duration of treatment are not taken into consideration.

Group 3: Postmenopausal patients irrespective if participating in randomized trials or not (n = 440)
This mixed group of patients were investigated because no control group (not treated with adjuvant TAM) was available in the randomized clinical trial involving postmenopausal patients (Group 2, above). Of the 440 patients in Group 3, 244 (55%) were also included in Group 2 above and 46 (10%) patients had participated in a previous multicenter randomized clinical trial where adjuvant TAM therapy (30 mg daily) for one year (with or without postoperative radiotherapy) was compared with no adjuvant systemic treatment (only radiotherapy [4]. The remaining 150 (34%) patients had not participated in randomized trials.

Follow up
All patients in the clinical trials were followed according to a strict protocol including clinical examination, mammography, and X-ray. Local and regional recurrences were verified by cytological or histopathological examination. A diagnosis of distant recurrence was based on unequivocal X-ray findings. Patients under 75 years of age, not participating in clinical trials, were followed according to a similar strict protocol, whereas those over 75 were followed more individually.

The median duration of follow-up for the three groups was as follows: All patients, 40 months; Group 1, 36 months; Group 2, 42 months; and Group 3, 41 months.

Laboratory assays

ER and PgR analysis
ER and PgR were measured with two different techniques, ER content with isoelectric focusing in polyacrylamide gels (IF) or enzyme immuno assay (EIA), and PgR content with the dextran-coated charcoal method with Scatchard analysis (DCC) or EIA [21–23]. In a comparison of previous results obtained with different ER and PgR assays in the same breast cancer samples [22, 23], we found inter-assay agreement to be satisfactory both for ER content (Spearman's rank correlation, $r_s = 0.98$; $n = 127$) and for PgR content ($r_s = 0.88$, $n = 97$), though somewhat higher values were obtained with EIA than with IF or DCC. Thus, the cut-off values adopted for defining receptor positivity had to be adjusted according to the measuring technique used. Samples with ER and PgR concentration values ≥ 10 fmol/mg protein obtained with IF and DCC were classified as positive, and samples with values below this level as negative [16]. The corre-

sponding level for EIA was 25 fmol/mg protein. The receptor data, covering a period of 10 years and including about 4,000 samples from 15 different hospitals, have shown satisfactory stability [24].

To investigate the importance of different PgR positive concentration levels, samples with PgR concentrations above or equal to 25 and below 200 fmol/mg protein were classified as low positive, whereas those with concentration values above or equal to 200 fmol/mg protein were classified as high positive. Of the 647 PgR+ samples, 292 (45%) belonged to the low positive concentration group and the remaining 355 (55%) of the patients to the high positive concentration group. As almost all samples in the present series were ER-positive, 200 fmol/mg protein was also used as cut-off for ER, yielding 48% below and 52% above or equal to this value.

Flow cytometric (FCM) DNA analysis

The samples were prepared for FCM DNA analysis in a one-step procedure previously described [25, 26] with slight modifications [27] as outlined in the following. Briefly, tumor tissue (100–200 mg) was thawed in 100–200 µl of citrate buffer (sucrose 250 mM, trisodium citrate 40 mM, dimethylsulfoxide 5%, pH 7.6) containing chicken and trout red blood cells (CRBC and TRBC, together 10^6 cells/ml). To enhance cell elution, the tissue was mechanically disintegrated with two forceps, after which 1–2 ml of nuclear isolation medium (NIM) containing propidium iodide (PI) was added (50 µg PI/ml, SIGMA P-5264; RNAse 0.1 mg/ml, SIGMA R-5125; Nonidet P 40 0.6% (v/v, SIGMA N-3516) in isotonic buffered saline, GIBCO). The samples were filtered (50 µm) and incubated in the dark for 10 min at room temperature, and then kept at + 4° C until required for FCM analysis, which was performed within one hour in an Ortho cytofluorograph 50 H as previously described [27].

Calculation of DNA index (DI). The modal values of the CRBC and TRBC G0/G1 peaks were used for zero-point adjustment of the DNA histogram [28]. The mean channel numbers of all G0/G1 peaks were corrected using the value obtained by zero-point adjustment, the resultant being then used for the calculation of DI with TRBC as the reference standard.

Definition of ploidy status. Tumors were defined as either DNA diploid (with one cell population – G0/G1 peak) or DNA non-diploid (with two or more cell populations – G0/G1 peaks) [29].

Calculation of SPF. The SPF was calculated planimetrically [30] assuming the S-phase compartment to constitute a rectangular distribution between the modal values of the G0/G1 and G2 peaks. In cases of bimodality in the 2C region and where the DI for the non-diploid cell population was below approximately 1.3, a mean SPF value was calculated for the diploid and near-diploid together. SPF was calculated exclusively in the non-diploid stemline when DI exceeded 1.3, and if the corresponding G2 peaks were distinctly separated. SPF was calculated in the most prominent non-diploid stemline in cases with two or more non-diploid peaks. Although no correction was made for background debris, SPF was not calculated when background debris predominated in the SPF region(s) of the histogram. SPF was not calculated if the corresponding G2 peak in the histogram could not be identified, or when the non-diploid stemline was small (G0/G1 < 10% of the total number of observations). In the present series, SPF was estimated in 647 (95%) of the 681 tumors analyzed for DNA with FCM.

Diploid tumors with an SPF ≥ 7.0% and non-diploid tumors with an SPF ≥ 12% were classified as high SPF, whereas the remaining tumors were classified as low SPF [31].

The coefficient of variation. The mean coefficient of variation (CV-value) for the diploid G0/G1 peak of 603 consecutive breast cancer samples at our laboratory was 3.2 ± 1.0 [31].

Statistics

Fisher's exact test (two-sided) was used to compare differences in prognostic factors between TAM-treated and not TAM-treated patients. The Kaplan-Meier estimate [32] was used to describe recurrence

Table 1. Recurrence-free survival according to uni- and multi-variate analysis (Cox's proportional hazard model) of prognostic factors in the group of patients a) not treated with TAM (n = 239), b) treated with TAM (n = 392), and c) all patients (n = 631)

Covariate	Univariate	Multivariate		
	p-value	p-value	RR[a]	95% confidence interval
a) Patients not treated with TAM				
Lymph node status	< 0.0001[b]			
1–3 *vs.* 0		0.005	2.4	1.3–4.4
4+ *vs.* 0		< 0.001	6.6	3.5–12.6
S-phase fraction				
high *vs.* low	< 0.0001	< 0.001	3.6	2.2–6.1
PgR+ concentration				
≥ *vs.* < 200 fmol/mg protein	NS	0.069	0.6	0.4–1.0
Tumor size				
> *vs.* ≤ 20 mm	0.015	NS		
ER concentration				
≥ *vs.* < 200 fmol/mg protein	NS	NS		
Ploidy status				
diploid *vs.* non-diploid	0.025	NS		
Menopausal status				
pre- *vs.* post-menopausal	NS	NS		
b) Patients treated with TAM				
Lymph node status	0.0021[b]			
1–3 *vs.* 0		0.006	2.9	1.4–6.1
4+ *vs.* 0		< 0.001	4.6	2.1–10.2
Tumor size				
> *vs.* ≤ 20 mm	0.0054	0.002	2.8	1.5–5.3
PgR+ concentration				
≥ *vs.* < 200 fmol/mg protein	0.039	0.020	0.5	0.3–0.9
ER concentration				
≥ *vs.* < 200 fmol/mg protein	NS	NS		
S-phase fraction				
high *vs.* low	0.055	NS		
Ploidy status				
diploid *vs.* non-diploid	NS	NS		
Menopausal status				
pre- *vs.* post-menopausal	NS	NS		
c) All patients				
Lymph node status	< 0.0001			
1–3 *vs.* 0		< 0.001	2.5	1.5–3.9
4+ *vs.* 0		< 0.001	4.8	2.9–7.9
S-phase fraction				
high *vs.* low	< 0.0001	< 0.001	3.5	2.1–5.9
Tumor size				
> *vs.* ≤ 20 mm	0.0015	0.003	1.8	1.2–2.7
Tamoxifen*SPF	–	0.005	0.4	0.2–0.7
PgR+ concentration				
≥ *vs.* < 200 fmol/mg protein	0.031	0.007	0.6	0.4–0.9
Tamoxifen				
with vs. without	0.0018	0.020	0.6	0.4–0.9
ER concentration				
≥ *vs.* < 200 fmol/mg protein	NS	NS		
Ploidy status				
diploid *vs.* non-diploid	NS	NS		
Menopausal status				
pre- *vs.* post-menopausal	0.05	NS		

[a] RR = relative risk.

[b] A log rank test companing recurrence-free survival in the three groups simultaneously.

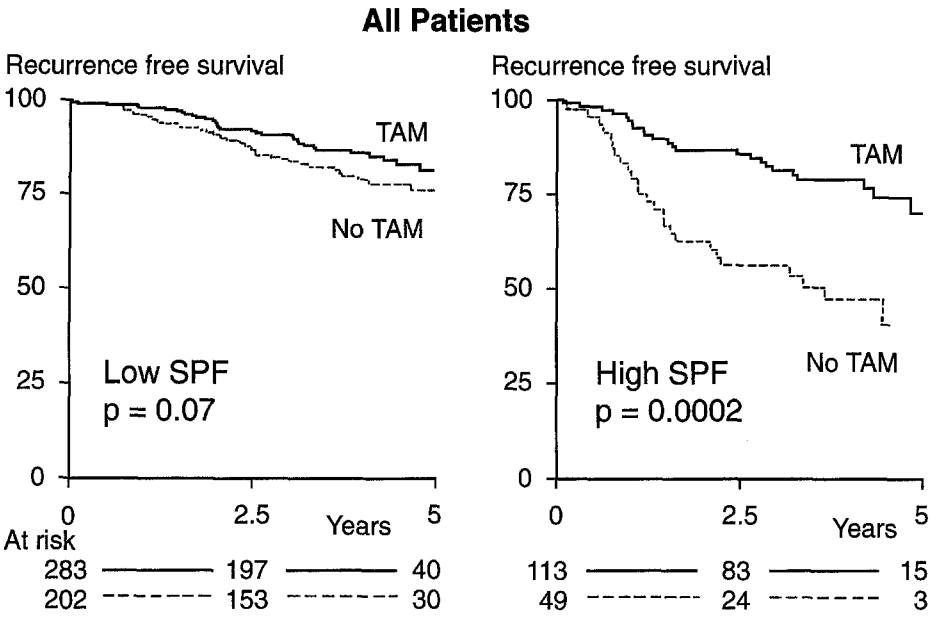

Fig. 1. Recurrence-free survival in relation to S-phase fraction and adjuvant tamoxifen for all patients in the present study (n = 647), irrespective of menopausal status or whether participating in randomized trials or not.

free survival and the log-rank test to compare survival in different subgroups [33]. The size of the population 'at risk' is given along the time axis. The estimate is drawn as long as at least five patients remain at risk, following the rule by Altman [34].

Cox's proportional hazards model was used for multivariate analyses [35]. The proportionality assumptions have been checked graphically, by plotting the log cumulative hazard for each level of one factor at a time versus time, and they do not seem to be grossly violated. This conclusion was verified using Schoenfeld's test for the final models presented in Table 1a–c [36]. No significant covariate-by-time interactions were found.

In order to compare the effect of adjuvant TAM between two separate subgroups (in this case low and high SPF), two Cox models were fitted. One model included SPF, treatment, and a set of other important covariates, and the other model also included the SPF* treatment interaction. The hypothesis: 'no difference in treatment effect' can in this setting be tested with a likelihood ratio test comparing the models with and without the interaction term.

Unless otherwise stated, p-values < 0.05 were considered significant.

Results

All patients

Among patients with PgR+ tumors, adjuvant TAM improved RFS ($p = 0.0026$), with a decrease in the 3 years recurrence rate from 22% to 12%. When SPF was also taken into consideration, TAM had a more pronounced effect for the high SPF subgroup ($p = 0.0002$; Fig. 1) than for the low SPF subgroup ($p = 0.07$), resulting in a decrease in the recurrence rate from 43% to 19% (high SPF) and from 17% to 9% (low SPF). The difference in treatment effect between the two SPF subgroups was statistically significant ($p = 0.005$, see below; multivariate analysis).

In the group of patients not treated with TAM, there was a striking difference in RFS between the low and high SPF subgroups ($p < 0.0001$). The corresponding p-value among patients treated with TAM was $p = 0.057$.

In our breast cancer material as a whole (n = 2598), the frequency of low SPF values is much higher among PgR+ tumors than among PgR– tumors (79% *vs.* 52%, $p < 0.0001$). Of the present series of PgR+ tumors, 75% had low SPF values.

Multivariate analysis. Among patients not treated with TAM, lymph node involvement and SPF were independent prognostic factors vis-à-vis RFS, whereas tumor size, the level of ER (low positive *vs.* high positive), DNA ploidy and menopausal status were not (Table 1a). The level of PgR (low positive *vs.* high positive) was near 'the borderline of significance' ($p = 0.069$).

Among TAM-treated patients, lymph node involvement, tumor size, and PgR concentration level were independent factors, whereas ER concentration level, SPF, DNA ploidy, and menopausal status were not (Table 1b).

In order to compare the effect of adjuvant TAM between the low and high SPF subgroups, a third multivariate analysis including all patients was performed. The same set of factors as above was tested plus treatment and interactions between treatment and these factors (see Material and Methods). The analysis demonstrated that lymph node involvement, tumor size, PgR level, SPF, TAM treatment, and also the interaction between TAM and SPF were independent prognostic factors, whereas menopausal status, ER level and ploidy status were not (Table 1c). The finding that the interaction factor (TAM*SPF) was an independent prognostic factor suggests that the effect of adjuvant TAM dif-

Table 2. Tumor and patient characteristics for the three different groups including patients with PgR+ and SPF-analyzed tumors, and with information on adjuvant tamoxifen treatment and follow up data

	All patients		Group 1		Group 2	Group 3	
	TAM	no TAM	TAM	no TAM	TAM	TAM	no TAM
n	396	251	65	65	244	321	119
years, median	63	51	46	46	66	66	71
Lymph node status							
% N0	30	50	29	22	30	30	61
% N1–3	48	30	48	52	48	48	18
% N4+	21	15	23	26	21	21	11
unknown	1	5	0	0	1	1	10
Tumor size							
mm, median	25	20	23	24	24	25	20
% ≤ 20 mm	35	51	34	40	37	35	60
% > 20 mm	65	49	66	60	63	65	40
ER							
fmol/mg protein							
median	250	148	67	76	290	300	380
negative, %	6	8	14	17	4	4	2
positive, %	94	92	86	83	96	96	98
< 200 fmol/mg protein, %	42	58	78	91	35	34	29
≥ 200 fmol/mg, %	58	42	22	9	65	66	71
PgR							
fmol/mg protein							
median	220	250	210	210	210	220	250
< 200 fmol/mg protein, %	46	44	48	48	49	46	45
≥ 200 fmol/mg, %	54	56	52	52	51	54	55
Ploidy status							
diploid, %	42	47	40	46	44	42	61
non-diploid, %	58	53	60	54	56	58	39
SPF							
median	6.8	5.6	7.2	5.6	6.7	6.8	5.4
low, %	71	80	71	71	72	72	86
high, %	29	20	29	29	28	28	14

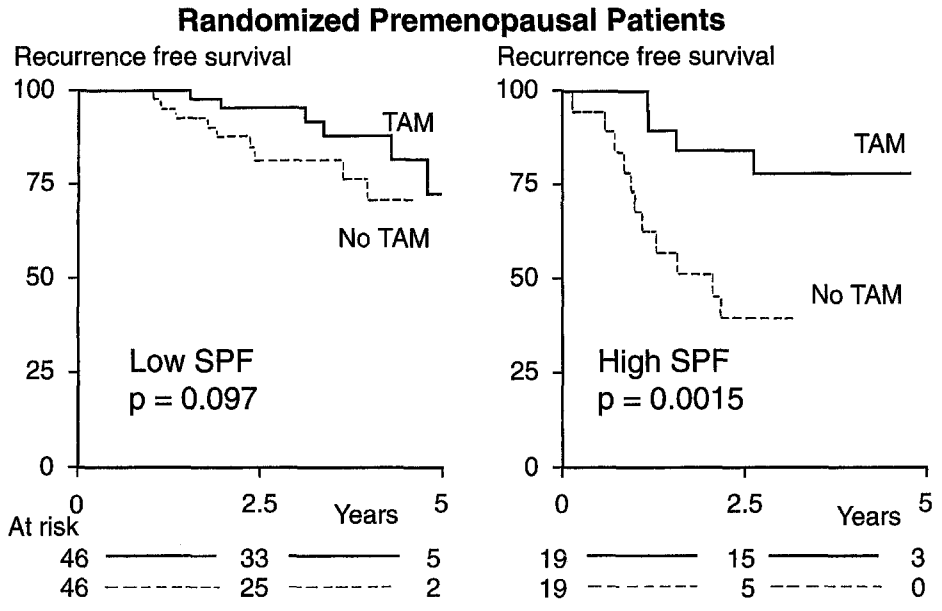

Fig. 2. Recurrence-free survival in relation to S-phase fraction and adjuvant tamoxifen for premenopausal stage II patients in a controlled randomized trial (n = 130).

fered between the low and high SPF subgroups, being more pronounced in the latter subgroup.

Subgroup analysis

The distribution of the different clinical and biological variables in the three different groups of patients in relation to adjuvant TAM treatment is shown in Table 2. In Group 1, none of the variables manifested statistically significant difference between TAM-treated patients and non-TAM-treated patients. In Group 3, another pattern was found: patients treated with adjuvant TAM manifested significantly higher lymph node involvement ($p < 0.0001$), larger tumors ($p < 0.0001$), and higher S-phase values ($p = 0.003$) than those not treated with TAM.

Group 1: Premenopausal patients in a randomized trial. TAM-treated patients with PgR+ tumors manifested significant improvement in RFS ($p = 0.0010$), thus confirming the results from a previous study [6]. In the PgR+ subgroup, a beneficial effect of adjuvant TAM was demonstrated for both the low and the high SPF subgroups (Fig. 2; $p = 0.097$ *vs.* $p = 0.0015$), resulting in decreases of the 3 year recurrence rate among TAM-treated patients from 60% to 22% (high SPF subgroup) and from 18 to 4% (low SPF subgroup). Although there was a clear tendency of a difference in effect after adjuvant TAM between low and high SPF subgroups, this difference was not statistically significant ($p = 0.068$). As can also be seen from Fig. 2, the prognostic importance of the SPF level was evident among non-TAM-treated patients, the 3 year recurrence rate being 18% in the low SPF subgroup and 60% in the high SPF subgroup ($p < 0.0001$). Among TAM-treated patients, there was no statistically significant difference in RFS between the high and low SPF subgroups ($p = 0.48$).

Group 2: Postmenopausal patients in a randomized trial. As all patients in this group received adjuvant TAM (for two or five years), the effect of this treatment in relation to a control group could not be investigated. In agreement with the results obtained for Group 1, no difference in RFS between the low and high SPF subgroups was found (recurrence rates 9% *vs.* 8%; Fig. 3; $p = 0.83$).

Group 3: Postmenopausal patients, both in and out-

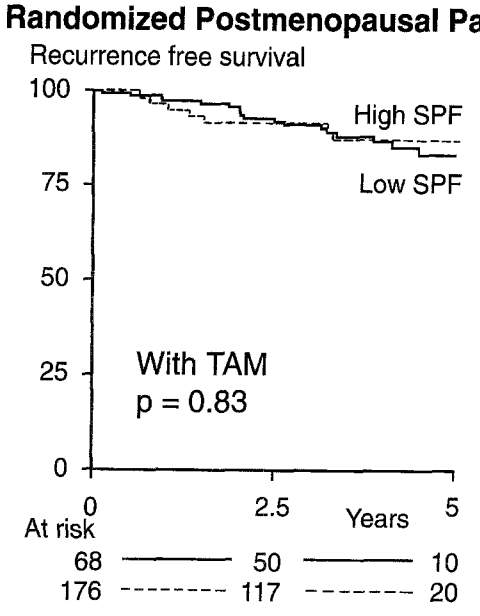

Fig. 3. Recurrence-free survival in relation to S-phase fraction for postmenopausal stage II patients treated with adjuvant tamoxifen in a controlled randomized trial (n = 244).

side randomized trials. As in the premenopausal group, a beneficial effect of adjuvant TAM was seen (although it was not statistically significant) for both the low (p = 0.17) and high SPF subgroups (p = 0.082; Fig. 4), the 3 year recurrence rates decreasing from 15 to 10% (low SPF subgroup) and from 29 to 17% (high SPF subgroup).

Discussion

In agreement with findings in previous studies by our group, adjuvant TAM was demonstrated to have a beneficial effect among breast cancer patients with PgR+ tumors [4, 6]. When the SPF (low *vs.* high) was also taken into consideration, the beneficial effect was more pronounced in the group of patients with high SPF tumors than in the group with low SPF tumors (p = 0.005; all tumors were PgR+). This pattern was consistent both in the series as a whole, and in subgroup analysis where the importance of selection bias was considered by including only patients participating in controlled clinical randomized trials. Moreover, similar results were obtained for both pre- and post-menopausal patients.

The relatively small TAM-induced improvement in RFS among patients with PgR+ and low SPF tumors may be explained by the fact that such patients already have a favorable prognosis – among the lymph node negative subgroup even comparable with overall survival in an age-matched control

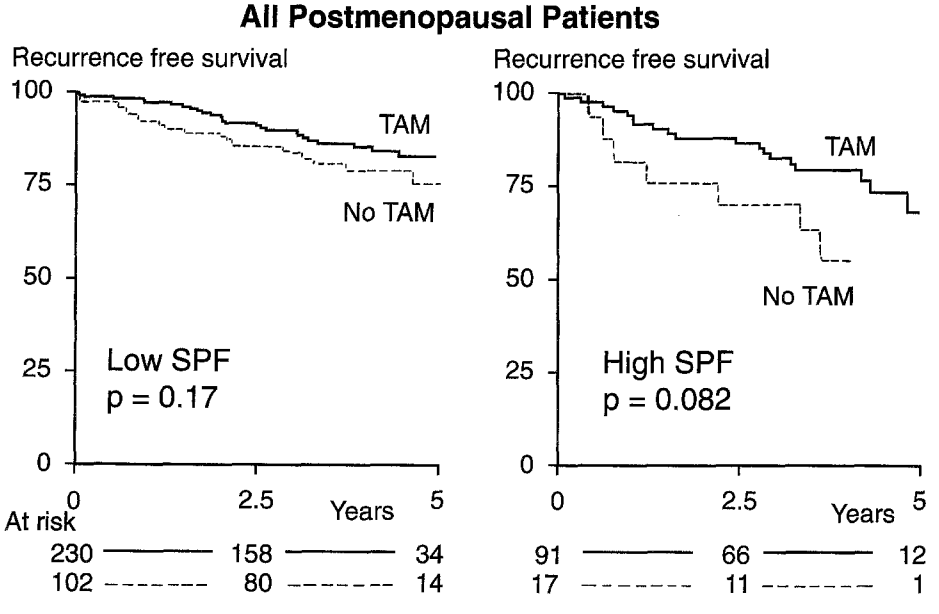

Fig. 4. Recurrence-free survival in relation to S-phase fraction and adjuvant tamoxifen for postmenopausal patients (n = 440).

group of healthy women [16]. Accordingly, it would be difficult to further improve RFS among such low-risk patients. However, although no statistically significant TAM-induced improvement in RFS was obtained, there was a clear tendency that adjuvant TAM had a beneficial effect also for patients with PgR+ and low-SPF tumors.

This pattern was similar for both pre- and postmenopausal subgroups, though more pronounced in the former. The more pronounced effect of tamoxifen for premenopausal patients in Group 1 than for postmenopausal patients in Group 3 is probably to be explained by the fact that patients in Group 1 were all participants in a controlled randomized clinical trial, as compared with only about two thirds of the postmenopausal patients in Group 3. The importance of this difference is illustrated in Table 1, from which it can be seen that in Group 1 (premenopausal patients) the TAM-treated and non-TAM-treated groups manifested similar tumor characteristics. By contrast in Group 3 (postmenopausal patients) those treated with adjuvant TAM tended to have breast cancer of more advanced stage and higher S-phase values than the non-TAM-treated control group.

Among premenopausal patients not treated with adjuvant TAM, there was a pronounced difference in RFS between the low and high SPF subgroups. These findings confirm previous results that SPF is a strong prognostic factor [18]. A noteworthy finding was that the difference between the low and high SPF subgroups was not statistically significant when adjuvant TAM was given, a finding confirmed in multivariate analysis where SPF was found to be an independent prognostic factor for RFS among patients not treated with adjuvant TAM, but not in the TAM-treated group. Therefore, a prognostic factor should be evaluated in a series of patients not given any adjuvant treatment. If some sort of systemic treatment (endocrine or cytotoxic) is given it is also the predictive value of a certain factor in relation to the given systemic treatment which is studied. Finally, among patients treated with TAM, it was not only the presence of PgR but also the PgR level that was important, as in multivariate analysis, PgR level (≥ 25 and < 200 fmol/mg protein *vs.* ≥ 200 fmol/mg protein) was found to be an independent factor.

The relationship between proliferation and endocrine therapy has previously been investigated in a small study of locally advanced primary, or local or distant recurrent breast cancer [11]. The hormone sensitivity of ER-positive breast cancer seemed here to be dependent on the rate of tumor cell proliferation, tumors with high levels of Ki67 staining (n = 13) rarely responding to therapy [11]. This result is in contradiction to ours, which might be explained by the fact that our study concerned adjuvant TAM, whereas theirs was not only based on treatment of more advanced breast cancer but was also carried out in a small series. TAM has been shown *in vitro* to be an antiproliferative agent, as indicated by the arrest of cells in the G0/G1 phase after treatment [37–39]. TAM has also been shown to reduce the Ki67 staining and thymidine labelling index in human breast cancers in [40, 41].

By way of control (data not shown), we investigated the effect of adjuvant TAM among patients with PgR negative tumors (n = 546), also taking the SPF level into consideration. Neither in the low nor the high SPF subgroup was any significant beneficial effect of TAM found. The same results were obtained for both pre- and post-menopausal patients.

To sum up, adjuvant TAM has been shown to have a beneficial effect on recurrence-free survival among breast cancer patients with PgR+ tumors, both in the low and high SPF subgroups, though the effect was more pronounced in PgR+ tumors with a high SPF.

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