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Original Paper

S-phase Fractions of Breast Cancer Predict Overall and Post-relapse Survival

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We studied the correlation of S-phase fraction (SPF) with clinical outcome in 127 pre- or perimenopausal patients with breast cancers treated by neoadjuvant chemotherapy from October 1986 to June 1990. When the patients were analysed using the median value of the SPF as a threshold, there was a small but non-significant difference in favour of low SPF tumours for metastasis-free survival. SPF was the only parameter predicting overall survival in multivariate analysis ($P < 0.002$) which included T, N, histopathological grade and steroid hormone receptors. The results of metastasis-free survival contrasted with previous analyses with shorter follow-up, so we tested the time-dependent influence of SPF on prognosis. It was thus shown that SPF significantly predicts metastasis-free survival only during the first 30 months, whereas the relative risk of cancer-related death according to SPF remains significant for 56 months. In order to find an explanation for the difference in predictivity between metastasis-free survival and overall survival, we studied the post-relapse survival. Significantly shorter survival (median 12 months) was associated with tumours presenting pre-treatment high SPF values, compared to the low SPF group for which 60% of the patients were still alive after 30 months of metastasis phase ($P = 0.002$). Our current results, in a homogeneous series with a median follow-up of over 5 years, emphasise the importance of proliferation-related parameters for breast cancer management.

Key words: breast cancer, neoadjuvant chemotherapy, response, S-phase fraction, prognosis

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INTRODUCTION

THERE IS a general consensus on the utility of chemotherapy as part of the multimodality treatment of breast cancer, especially in premenopausal patients. The results of the meta-analysis of the 10-year survival of operable breast cancers, having received adjuvant chemotherapy, has shown a survival advantage of the order of 10% in favour of adjuvant chemotherapy [1]. From these results, it has been concluded that survival is improved through the effect of chemotherapy on clinical micrometastases, already present in a substantial proportion of patients at the time of diagnosis [2, 3]. Some experimental data showed a benefit in administering chemotherapy prior to locoregional treatment [4, 5] and soon this modified sequence, called neoadjuvant chemotherapy, was applied to locally advanced or inflammatory breast cancers [6, 7]. Neoadjuvant chemotherapy has been incorporated as a part of a breast conserving approach for large tumours that would otherwise be treated by total mastectomy [8], as demonstrated by Bonnadonna and associates for tumours of less than 7 cm [9].

Neoadjuvant chemotherapy was introduced in our institution in the early 1980s and two randomised trials were conducted in order to define the value of this modified sequence [10, 11]. DNA flow cytometry was initiated in 1986 on cytological fine-needle samples obtained at diagnosis [12]. Thus, the results on DNA content and S-phase

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fraction (SPF) are available at diagnosis and their relationship with the therapeutic efficacy can be studied. We report here on the impact of SPF pretreatment values on clinical outcome.

PATIENTS AND METHODS

Cytological diagnosis of breast cancer is the general rule for non-surgically treated patients in our institute. Our study of SPF in relation to neoadjuvant chemotherapy was initiated concomitantly with a trial conducted in our institute from October 1986 to July 1990 [11]. Eligible patients had tumours classified as T2 of more than 3 cm or T3 of less than 7 cm, N0 or N1, M0 and were pre- or perimenopausal. One hundred and twenty-five cytological fine-needle samples, corresponding to patients entered in the neoadjuvant chemotherapy arm of the trial, were sent to the laboratory for DNA flow cytometric analysis. There were 120 interpretable histograms, of which 93 (78%) were suitable for SPF measurement. Before the current analysis, a search was conducted in our central data bank for patients of similar age that were not randomised in the trial. Only cases corresponding to tumours of similar clinical definition treated by an identical neoadjuvant chemotherapy protocol during the same period were retained. Thus, an additional 34 patients with known SPF values were included in the study.

Neoadjuvant chemotherapy consisted of four cycles of CFA (cyclophoshamide 500 mg/m^2 on days 1 and 8, 5-fluorouracil 500 mg/m^2 on days 1, 3, 5 and 8 and doxorubicin 25 mg/m^2 on days 1 and 8). The subsequent locoregional treatment, including irradiation or local excision followed by radiotherapy, was determined by the extent of the clinical response.

Sample preparation and data collection were as previously described [13, 14].

Overall survival and metastasis-free survival were calculated from the first day of treatment. Survival curves were estimated by means of the Kaplan–Meier method and compared using the logrank test. In case of discrepancy, the Breslow test was also used; this test is more sensitive to early events since it weighs the number of events by the total number of patients at risk.

Prognostic influence of the various parameters was assessed using the Cox proportional hazards method. The variation in the prognostic significance of SPF with time was based on a time-dependent relationship between hazard and covariates [15].

The hazard function was $h(t) = h_0(t)\ \exp\ (bZ + b_1 Z_1(t))$, where Z is the indicator variable of SPF, i.e. less or more than 5% and $Z_1(t) = Z^*$ function of time, which was chosen as $f(t)$ = time.

If no significant deviation from the proportional hazards is observed, then the ratio for the two groups is constant over time and b_1 should be close to 0. In the case of a significant deviation, b_1 will be positive for an increasing risk with time and negative for a decreasing prognostic influence of SPF with time.

RESULTS

SPF values at diagnosis, measured on DNA histograms after flow cytometrical analysis of fine-needle cytological samples, were available for 127 primary breast cancer patients treated by neoadjuvant chemotherapy. Their mean age was of 45 years (range 28–56) with a median follow-up of 63 months (range 21–96). During the time of observation, 39 metastases had occurred and 23 patients had died. The clinicopathological and biological characteristics of the series are summarised in Table 1, together with metastasis-free and overall surviving fractions at 3 and 5

Table 1. *Clinicopathological and biological characteristics of the primary tumours together with univariate prognostic analysis*

		Metastasis-free survival			Overall survival		
Parameter	*n*	3 years (%)	5 years (%)	Logrank	3 years (%)	5 years (%)	Logrank
Age (years)							
<35	21	63	50	$P = 0.13$	81	73	$P = 0.09$
⩾35	106	81	71		92	86	
Size (T)							
T2	93	85	73	$P = 0.06$	94	88	$P = 0.08$
T3	34	62	58		85	75	
Nodal status							
N1	74	85	78	$P = 0.04$	93	88	$P = 0.03$
N0	53	70	55		88	80	
OR							
+	62	79	68	$P > 0.80$	95	89	$P > 0.45$
–	52	79	72		86	82	
PgR							
+	54	74	66	$P > 0.45$	96	86	$P > 0.8$
–	59	81	74		86	85	
SBR grade*							
I	16	88	77	$P > 0.6$	93	93	$P > 0.5$
II	50	77	64		94	86	
III	27	70	66		82	72	
SPF							
<5%	64	84	75	$P = 0.1$	100	95	$P < 0.002$
⩾5%	63	71	62		82	74	

*Some data missing.

OR, oestrogen receptor. PgR, progesterone receptor.

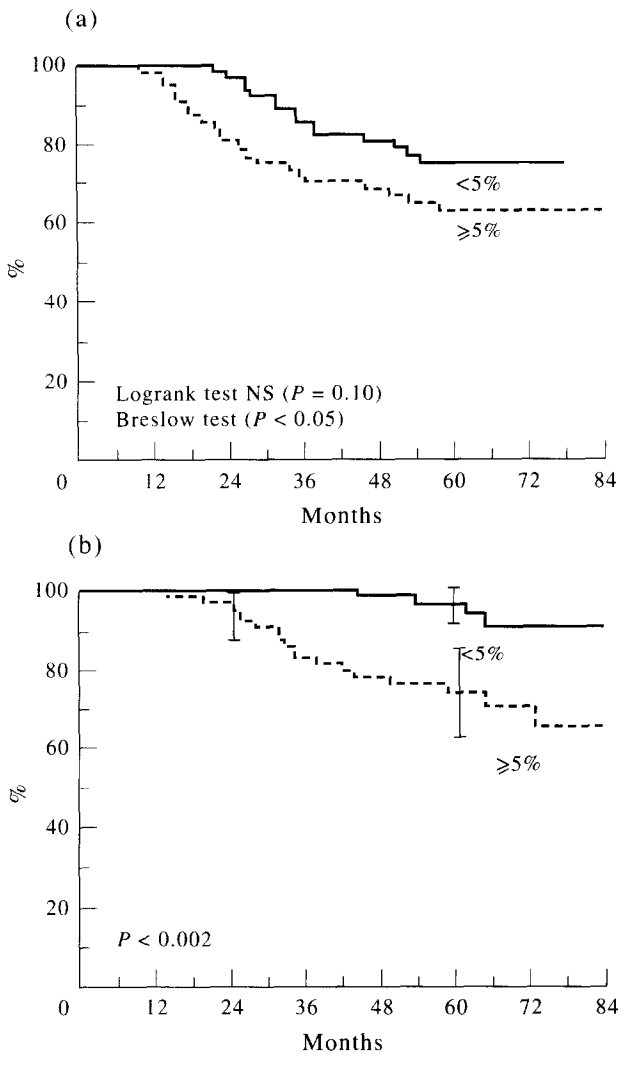

Figure 1. Actuarial metastasis-free (a) and overall (b) survival curves according to pretreatment SPF value. The median value (5%) was used as a cutoff. Both logrank and Breslow test results are shown for the metastasis-free survival curve.

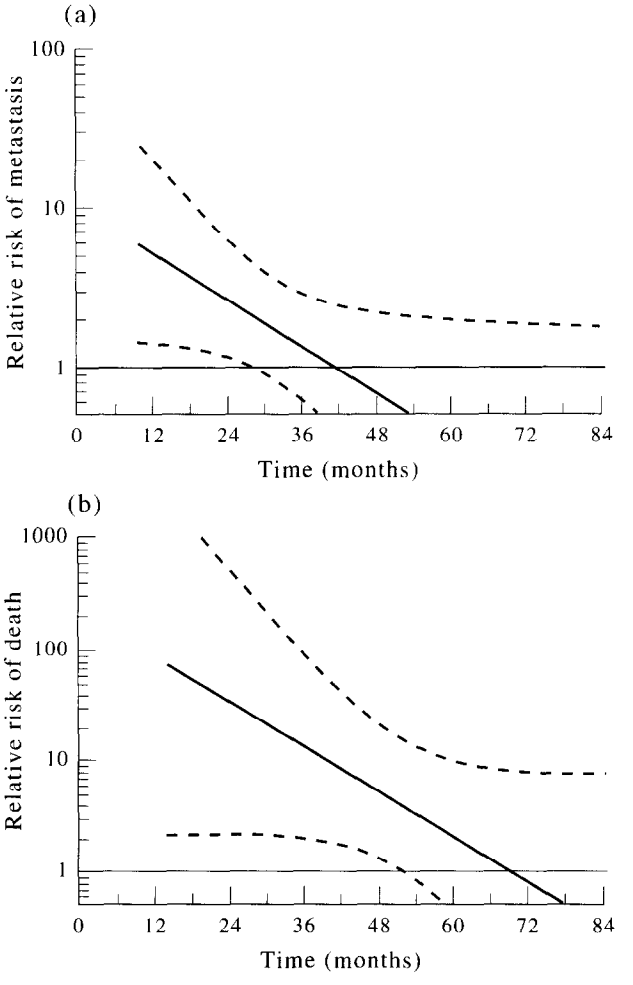

Figure 2. Curves showing the evolution of relative risk for metastasis (a) or cancer-related death (b) with time. The relative risks are calculated at time points corresponding to events. Deviation from the proportional hazards model is tested as a function of time according to the formula indicated in the Patients and Methods section, resulting in a curve (solid line) plotted in semi-log scale with 95% confidence intervals (broken lines). A horizontal line is drawn at relative risk = 1, so that its intersection with the lower boundary of the 95% interval of the relative risk curve indicates the time at which SPF ceases to have a significant influence on prognosis.

years. Clinical node involvement but not SPF predicted metastasis. Nevertheless, as shown in Figure 1, a low SPF was associated with significantly delayed metastases (Breslow test; $P < 0.05$), even if the effect disappeared with time (logrank test; $P < 0.10$). SPF predicted overall survival with a high significance ($P < 0.002$), while clinical node status was less significant ($P < 0.04$). In multivariate analysis, SPF was the only parameter to predict survival (relative risk of 4.3, range 1.6–11.7, $P < 0.0002$) and lymph node status alone predicted metastasis-free survival (relative risk of 2.2, range 1.1–4.1, $P < 0.04$).

Since 1992, a number of intermediate analyses have been performed on this group of patients and high SPF was found to correlate with shorter metastasis-free survival in all of them [16, 17]. This observation led us to introduce a time-dependent variable in the model to verify the proportional hazards function. In both cases, there was a significant deviation ($P < 0.05$ for survival and $P < 0.03$ for metastasis), confirming a decreasing relative risk of distant relapse or death over time. Taking into account this time effect, high SPF was significantly related to early metastasis ($P < 0.008$) or death ($P < 0.003$), although the relationship between SPF and prognosis lasted longer for overall survival (56 months) than for metastasis-free survival (30 months). Figure 2a,b illustrates the relative risk of event and its 95% confidence interval, taking into account the decrease with time. Following this observation, a time-dependent factor for SPF was introduced in the Cox model for metastasis-free survival. In this instance, SPF was retained in the Cox model as significant even when adjusted on nodal status ($P < 0.05$).

The relative discrepancy between the results of metastasis-free survival and overall survival was further investigated through the influence of SPF on post-relapse survival. In Figure 3 are shown the actuarial survival curves for low and high SPF patients, calculated as of the discovery of metastasis (a total of 39 patients). The difference was highly significant (relative risk 3.9, $P < 0.002$), with patients in the low SPF group having a survival of 58% at 30 months (5 patients at risk) compared to 18% for the patients in the

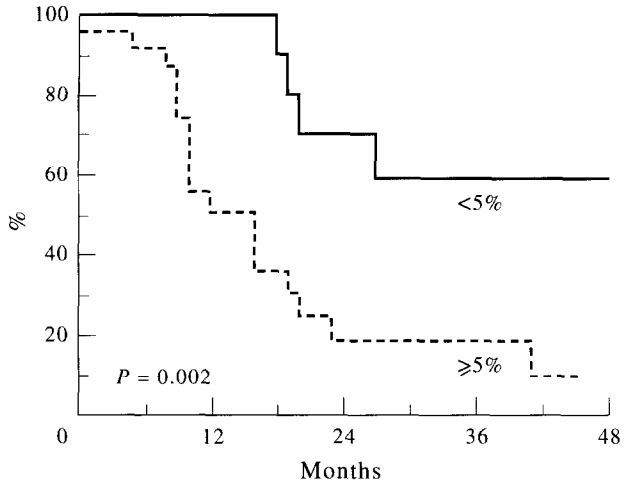

Figure 3. Kaplan–Meier curves for post-relapse survival acording to SPF. The curves correspond to 39 patients for which metastasis was diagnosed after the beginning of treatment.

high SPF group. Oestrogen receptors were marginally related to post-relapse survival, but only SPF was retained in multivariate analysis. Because of the consistently observed strong correlation between response to neoadjuvant chemotherapy and high SPF [13, 16], we analysed the differences in post-relapse survival according to initial response. Observed versus expected ratios and surviving fractions at 24 months for the four groups are shown in Table 2. There was no difference between major and minor responders in the low SPF group ($P = 0.72$), while a small but non-significant difference was observed in favour of the high SPF major responders ($P = 0.17$), which was somewhat intermediate. SPF was highly significantly prognostic among minor responders ($P < 0.004$) and less so for major responders ($P < 0.05$).

DISCUSSION

Proliferative activity of breast cancers was proposed as a significant prognostic factor 15 years ago [18, 19]. Since then, a large body of data has accumulated in favour of the relationship between increased proliferation measured by different methods [20–25] and decreased survival. However, in particular, when SPF was measured on DNA histograms obtained by flow cytometry, the literature was shown to contain a number of discrepancies, some of which were attributed by a panel of experts to technical reasons [26, 27]. It must be noted that, with the exception of the studies on cancers with no lymph node involvement, all other series were highly heterogeneous in terms of clinical stage and treatment [28]. This observation led Kute and associates [29] and Witzig and associates [30] to suggest that the use of chemotherapy reduced the prognostic impact of DNA ploidy or proliferative activity of breast cancers.

In a preliminary report on 55 patients, we previously showed the existence of a relationship between a high SPF value and increased clinical response to neoadjuvant chemotherapy [13], which we subsequently confirmed repeatedly on larger samples [16, 31]. Such a relationship has been reported in other studies of metastatic breast cancer [32], or in neoadjuvant chemotherapy-treated primary breast cancer patients [33–35]. Although there is a growing body of evidence, associating high proliferation with response to chemotherapy, only few studies have analysed the impact of proliferation on the outcome in the adjuvant setting, indicating a preferential improvement in the case of rapidly proliferating primary tumours [2], or a more pronounced improvement in the high SPF group [36, 37].

In the present study, after a long follow-up (median 63 months, range 21–98) of 127 patients, we report the highly significant association of SPF with overall survival. Since the population of interest was initially defined through clinical staging (sizes of 3–7 cm, clinical nodal status N0 or N1), it is not so surprising to observe that neither T nor N are highly significant predictors of survival; the same applies to histopathological grade and steroid hormone receptors. However, SPF was not significantly related to metastasis-free survival, whereas clinical node status was. This clearly contrasts with previous intermediate analysis of the same series [16]. Thus, we hypothesised a time-dependent prognostic value of SPF which could explain these differences. Not only was the time-dependency hypothesis verified, but, taking into account the decrease of the relative risk of metastasis over time, SPF was retained in multivariate analysis in addition to the clinical node status. Interestingly, a time-dependent significance was also observed for overall survival, but the significance lasted longer (4.5 years as opposed to 2.5 years for metastasis-free survival). The actual duration of the significance can only be held as indicative since the confidence intervals shown in Figure 2 are quite large.

A decrease in the significance of proliferation measurements as prognostic factors with time has been noted before. Stal and associates have reported that metastasis-free survival is related to SPF during the first 3 years [24], while Tubiana and associates have proposed that only the very low TLI group is of better prognosis in the long run [23]. Our series concerns a more homogeneous group of patients, treated in a uniform manner, giving new insights for the utility of proliferation measurements in the clinical management of breast cancers. As a biological indicator (tumour growth), SPF is also related to post-relapse survival. The contrast between this inherent property of biological parameters and clinical stage (size and nodal status), which in

Table 2. Post-relapse survival in relation to SPF and response

Group	Response	O/E	Survival at 2 years	Statistics
SPF < 5%	Major (7)	0.45	80%	$P = 0.72$
	Minor (9)	0.38	60%	
SPF ⩾ 5%	Major (13)	1.23	24%	$P = 0.17$
	Minor (10)	2.37	10%	

most cases predicts disease-free and overall survival but not post-relapse survival, has been emphasised before [38].

It is generally assumed that any parameter found to be initially related to disease-free or metastasis-free survival, with sufficient follow-up, will predict overall survival. Our results show that caution should be used for such interpretations. In the case of SPF, the discrepancy between the non-significant relationship with metastasis-free survival and the highly significant prediction of overall survival can be explained by the fact that only the early metastases are predicted by SPF, while patients with low SPF primary tumours seem to have improved survival at the metastatic phase. Steroid hormone receptors have been reported to predict post-relapse survival [39]. In our series, there was a trend for oestrogen receptor only in univariate analysis.

In the current context, with the proposed use of neoadjuvant chemotherapy as an integral part of a breast conserving strategy in operable breast cancer [9], our results seem to bring forward a number of questions concerning the use of the right systemic treatments depending on individual tumour behaviour. Neoadjuvant chemotherapy may not significantly benefit patients with slowly proliferating tumours. Indeed, the initial regression of the low SPF primary tumours does not predict disease-free interval [16], overall survival or even post-relapse survival. However, approximately 80% of the tumours with high pretreatment SPF will significantly regress during neoadjuvant chemotherapy and the corresponding patients can be offered breast conservation.

Treatment failures are much more frequent among high SPF non-responders (median overall survival 30 months), but even in this poor-prognosis group, patients that do not recur within the first 3 years following the initial diagnosis are actually long-term survivors. An obvious interpretation would be that there is no direct relationship between tumour proliferation and the presence of clinical metastases at diagnosis. A parameter directly related to metastatic potential is still to be uncovered.

In our experience of neoadjuvant chemotherapy, a more than 50% regression of the high SPF tumours is obtained within the first two cycles [40], a substantial proportion of these early partial responders continuing to complete response in the subsequent chemotherapy cycles. Thus, a consequence of our results would be to apply more aggressive regimens to these patients with non-responding tumours as early as possible.

Response to neoadjuvant chemotherapy has been found to predict locoregional or distal control in a series of 200 patients [40]. In our subset of 127 patients, significance was not reached, although there was a definite trend (logrank test $P < 0.09$, Breslow $P < 0.04$). Adjusting the prognostic significance of SPF to response, it was established that only the high SPF non-responders did significantly worse in terms of survival. If we are to improve the survival of the major responders further, additional prognostic information will be necessary to allow a better substratification. It is not our purpose to provide definite conclusions on the modifications of the treatment of stage II or IIIA breast cancer on the basis of a series of 127 patients. However, because of the strong interaction between tumour proliferation and chemotherapy, SPF analysis should be performed prospectively (ideally on fresh/frozen samples), within randomised trials, if we are to improve the efficacy of the available therapeutic modalities by using biological parameters for therapeutic decisions.

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