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Factors Affecting Relapse in Node-negative Breast Cancer. A Multivariate Analysis Including the Labeling Index

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Abstract—*Between* 1975 *and* 1982, 167 *patients with carcinoma of the breast without axillary lymph node metastases were studied. The thymidine labeling index (LI), representing the percentage of cells in the DNA synthesis phase, was measured in all these patients. High LI values were more frequently encountered in young patients* (P = 0.05), *in low estrogen receptor (ER) tumor content* (P = 0.007) *and in high grade tumors* (P = 0.0002). *The overall 8-year relapse-free survival (RFS) was* 68%. *Univariate analysis demonstrated that RFS was influenced by histological grading* (P = 0.03), *ER* (P = 0.03), *PR* (P = 0.02) *and LI* (P = 0.01). *Multivariate analysis using the Cox regression model selected the LI as the single significant prognostic factor with regard to RFS* (P = 0.037). *These results emphasize the important role of cell proliferation kinetics in defining node-negative breast cancer patients with a high risk of relapse.*

INTRODUCTION

BREAST CARCINOMAS without axillary lymph node metastases, also called node-negative breast cancer, are considered to have a good prognosis. However, the probability of relapse within 6 years reaches almost 30% [1, 2]. The clinical and histopathological prognostic factors remain unclear [3–6]. Recently, tumor cell kinetics were found to be an important prognostic indicator affecting outcome of breast cancer in general [7–9] and node-negative breast cancer in particular [1, 2]. Data concerning the prognostic value of hormone receptor levels are controversial and this may be due in part to the use of different threshold values for receptor positivity [10, 11].

In this study, we have extended our previous report in which the labeling index (LI) had a marked influence on overall survival and relapse-free survival (RFS) [2]. The sample size has been increased and the follow-up extended. The association between the LI and the other clinicopathological variables was examined. Their prognostic value was assessed in univariate and multivariate analysis to evaluate their independent influence on relapse. RFS rather than overall survival was chosen as an endpoint, as the latter parameter is largely affected by the response to treatment given after relapse.

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PATIENTS AND METHODS

Patients

One hundred and sixty-seven patients treated between 1975 and 1982 were included in the study. The median age at diagnosis was 60 years (range: 28–85). Menopausal women accounted for 75% (125/167) of the cases. These patients had their last menses more than 1 year prior to diagnosis. The left breast was involved in 97 cases vs. 70 for the right breast. The site of the tumor was in the outer quadrants in 45% of the cases, the others having either central or inner quadrant lesions.

Tumor size was measured in 162 patients. The median value was 30 mm, with a range of 10–80 mm. According to the TNM classification [12], 56 (34.5%) were classified as T1, 97 (60%) as T2 and nine (5.5%) as T3 tumors.

All patients were primarily treated with surgery. Of these, 116 (69.5%) had Patey mastectomy and 51 (30.5%) had partial mastectomy or lumpectomy. Axillary dissection was carried out in all patients. None of them had any lymph node metastasis on microscopic examination. The median number of lymph nodes dissected was 14. Patients treated conservatively were given radiotherapy: 45 Gy to

the breast, 60 Gy to the tumor bed. No adjuvant medical treatment was given until relapse.

Histological grading [13] was possible in 150 cases. Grade I was found in 53 tumors (35%), grade II in 69 (46%) and grade III in 28 (19%).

Hormone receptors

Hormone receptors were assessed by the dextran charcoal technique, described elsewhere [14]. Estrogen receptors (ER) were analyzed in 162 cases. The median value was 35 fmol/mg of cytosol protein, with a range of 0–1040 fmol/mg. Progesterone receptors (PR) were measured in 142 cases. The median value was 45 fmol/mg (range: 0–2350). Cut-off research studies [11] were undertaken in order to obtain the optimum ER and PR levels above and below which the RFS was most significantly different. This resulted in a limit of positivity of 20 fmol/mg for ER and 35 fmol/mg for PR.

Labeling index

The proportion of tumor cells in the DNA synthetic phase, called the labeling index (LI), was determined by using a technique described previously [2, 14]. Briefly, biopsy fragments were incubated for 30 min in a culture medium containing tritiated thymidine. They were then fixed, paraffin-embedded and sectioned. Slides were dipped in Ilford K2 emulsion and exposed for 21 days at 4°C. They were then developed, fixed and stained. Cells containing 5 grains or more were considered labeled. On the average, 1000 cells were scored per sample and three samples per tumor were examined. The labeling index was expressed as the number of labeled tumor cells × 100/the number of total tumor cells. The median LI was 2.14% with a range of 0.1–9.43%. Cut-off research studies have demonstrated that the median value adequately differentiated subpopulations having different prognoses [11].

Table 1 describes the population characteristics together with the LI values in each class.

Follow-up

Patients were examined twice a year for the first 5 years and yearly thereafter. Investigations consisted of chest X-ray, mammography, liver ultrasonography and alkaline phosphatase evaluation every year, and bone scintigraphy every other year. Following relapse, chemotherapy was given to pre- and paramenopausal patients, and hormonal therapy to postmenopausal patients.

Statistical analysis

The Spearman rank correlation coefficient and the Kruskal–Wallis one-way ANOVA test were used to analyze the association between variables. RFS curves were plotted using the Kaplan–Meier method [15] and statistical differences established by the log-rank test. A multivariate analysis using Cox's proportional hazard regression model [16] was carried out to assess the independent contribution of each variable to RFS.

RESULTS

The association between the LI and the other clinicopathological variables is summarized in Table 2. The LI was inversely correlated with

Table 1. Patient characteristics and relation to LI

	No. of cases (%)	Median LI (range)	No. of cases with above median LI (%)
All patients	167	2.14 (0.1–9.4)	83 (50)
Age:			
≤60	84 (50)	2.30 (0.1–8.9)	45 (54)
<60	83 (50)	2.01 (0.1–9.4)	38 (46)
Premenopausal	42 (25)	2.49 (0.5–8.9)	25 (30)
Postmenopausal	125 (75)	2.01 (0.1–9.4)	58 (70)
Tumor size (TNM):	162		80
T1	56 (35)	2.24 (0.3–9.4)	29 (36)
T2	97 (60)	2.14 (0.1–9.2)	48 (60)
T3	9 (5)	1.53 (0.8–6.4)	3 (4)
Grading:	150		76
Grade I	53 (35)	1.63 (0.1–5.7)	21 (28)
Grade II	69 (46)	2.08 (0.5–8.1)	33 (43)
Grade III	28 (19)	3.15 (0.3–9.4)	22 (29)
ER:	162		80
≤20 fmol/mg	64 (40)	2.47 (0.3–9.4)	37 (46)
>20 fmol/mg	98 (60)	1.88 (0.1–6.6)	43 (54)
PR:	142		73
≤35 fmol/mg	67 (47)	2.56 (0.1–9.4)	40 (55)
>35 fmol/mg	75 (53)	1.87 (0.5–7.6)	33 (45)

Table 2. The association between LI and other variables

	Spearman test	Kruskal–Wallis test	Significance
Age	$r = -0.151$		$P = 0.05$
ER	$r = -0.210$		$P = 0.007$
PR	$r = -0.134$		$P = 0.11$
Size (T1, T2, T3)		$\chi^2 = 0.73$	$P = 0.69$
Grade (I, II, III)		$\chi^2 = 16.9$	P = 0.0002

patient age: the younger the patient, the higher the LI. A similar type of correlation was observed between LI and ER, but not between LI and PR, despite the close correlation between ER and PR. Tumor size was not associated with LI. On the other hand, high LI values were more frequently encountered in high grade tumors.

ER but not PR was positively correlated with patient age ($P < 0.00001$). The percentage of ER-positive and PR-positive tumors decreased with the increase in histological grade ($P = 0.0034$ and $P = 0.0002$ respectively).

Forty-five relapses were observed during the follow-up period. The 8-year RFS was 68% (Fig. 1).

The following variables did not affect the disease outcome significantly: age, menopausal status, site of tumor or type of surgery. Tumor size did not affect the RFS either. For T1, T2 and T3 lesions, the respective values were 65, 72 and 59% ($P = 0.58$). It should be noted, however, that 95% of the tumors were T1 and T2 (Table 1).

RFS was influenced by the histological grade. The values were 79, 61 and 67% for grade I, grade II and grade III tumors respectively ($P = 0.03$). Patients with tumor ER content ≤20 fmol/mg had 55% RFS vs. 78% for patients with higher ER tumor level ($P = 0.03$). Likewise, RFS was affected by the level of PR. Patients with tumor PR content ≤35 fmol/mg had 65% RFS vs. 81% for patients with a higher level ($P = 0.02$).

With regard to LI, patients were classified into two groups according to the median LI value: 2.14%. Those with low tumor LI had 79% RFS vs. 56% for high tumor LI patients ($P = 0.01$) (Fig. 2). It is worth noting that no relapse occurred in the low LI group during the first 2 years. The relative risk of relapse at each level of the variables is given in Table 3.

Following this univariate study, a multivariate analysis was conducted to test the independent prognostic role of the size, grade, ER, PR and LI. The results are given in Table 4. When all these variables were taken into account, the LI was the best predictor of relapse. Through a stepwise analysis, the model selected the LI as the single independent prognostic factor regarding RFS. PR came next, short of significance, whereas ER, grading and tumor size were not of significant value once the other parameters were considered.

DISCUSSION

In a previous report on a smaller population, we have demonstrated that the LI affects the outcome of node-negative breast cancer [2]. The extension of the population to 167 patients allowed study of the association between the various parameters and made it possible to carry out a multivariate analysis.

In agreement with other studies, the cell proliferation kinetics were weakly related with patient age [17–19], more closely related to ER [14, 17, 18, 20–23] and to histological grading [21, 23]. In view of the association between ER, grading and LI, it was interesting to investigate the relative impact of each of them on the risk of relapse.

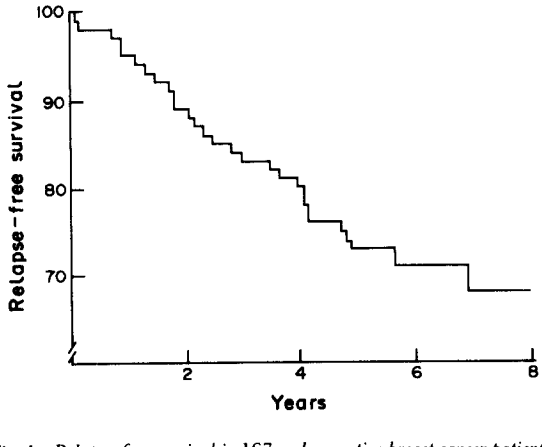

Fig. 1. Relapse-free survival in 167 node-negative breast cancer patients.

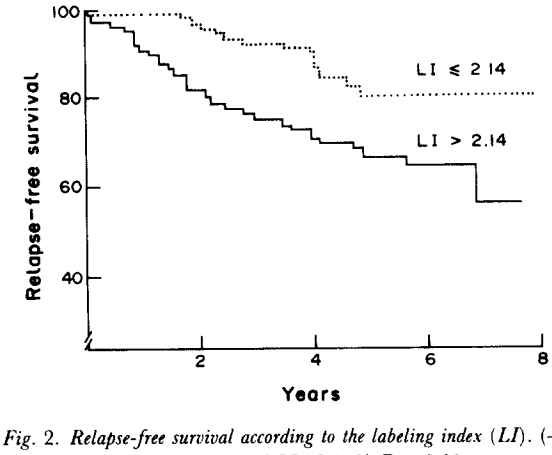

Fig. 2. Relapse-free survival according to the labeling index (LI). (—) LI>2.14%;(. . .)LI≤2.14%.P = 0.01.

Table 3. Relative risk of relapse established by a univariate analysis

	Risk ± S.D.	Significance
Size:		
T1	1	
T2	0.88 ± 0.27	$P = 0.58$
T3	1.25 ± 0.73	
Grade:		
I	1	
II	1.77 ± 0.37	$P = 0.03$
III	1.44 ± 0.49	
ER:		
>20 fmol/mg	1	$P = 0.03$
≤20 fmol/mg	2.53 ± 0.82	
PR:		
>35 fmol/mg	1	$P = 0.02$
≤35 fmol/mg	1.95 ± 0.42	
LI:		
≤2.14%	1	$P = 0.01$
>2.14%	2.83 ± 1.00	

Table 4. Results of multivariate analysis of prognostic factors with regard to relapse-free survival

			Stepwise analysis	
	Relapse risk	*P* value	Relapse risk	*P* value
Size:				
T1	1		—	
T2	1.15	0.66	—	
T3	1.32		—	
Grade:				
I	1		—	
II	1.11	0.68	—	
III	1.23		—	
ER:				
>20	1	0.60	—	
≤20	1.22		—	
PR:				
>35	1	0.12	1	0.061
≤35	1.83		1.98	
LI:				
≤2.14	1	0.07	1	0.037
>2.14	2.07		2.17	

In this series, RFS is comparable to that reported by others in node-negative breast cancer [1], suggesting no selection in our population. RFS was not affected by tumor size. It should be recalled, however, that our population was mainly composed of T1 and T2 tumors (Table 1). Many investigations conclude that tumor size does influence the risk of relapse of node-negative breast cancer [19, 24, 25]. Others do not find this factor significant [26–28] or observe a complex relationship [6].

The RFS was influenced by histological grade, a finding also reported by most [4, 5, 26, 29] but not all [30] investigators in node-negative breast cancer. However, when the other factors were taken into account in the Cox model, the grading lost its predictive power (Table 4), probably because of its association with LI (Table 1, 2).

The role of ER as a marker affecting the prognosis of breast cancer has been frequently studied. It is generally agreed that the risk of relapse depends, among other factors, on ER [19]. In one study, ER lost its predictive power when confronted with LI [24]. Recent data suggest that the currently used value of 10 fmol/mg as a threshold of positivity should be reevaluated [11, 31, 32]. It is likely that there is some sort of proportionality between the death rate and the level of hormone receptors [31, 32]. We have recently demonstrated that cut-off levels higher than 10 fmol/mg were better predictors of overall survival [11]. With regard to RFS, the optimum cut-off levels were 20 fmol/mg for ER and 35 fmol/mg for PR. In a univariate analysis, both receptors significantly affect RFS (Table 3). By considering the other factors in a multivariate analysis, both receptors lost their predictive power, PR being short of significance.

The role of cell kinetics in predicting the outcome of breast cancer is regaining interest, particularly with the advent of flow cytometry. Many studies have underlined the adverse influence of a high percentage of S-phase cells, whether defined by autoradiography, as in the present study, or by flow cytometry [1, 2, 7, 9, 17, 18, 22–24, 33]. Not only do cell kinetics affect RFS in univariate analysis, but LI stands as the single independent predictor of relapse, as determined by stepwise analysis, in agreement wih a previous report [24]. In a recent review, we have observed that in six out of seven multivariate analyses of prognostic factors of breast cancer in which cell kinetics were studied, this parameter kept an independent prognostic value (Tubiana and Courdi, submitted for publication).

In conclusion, histological grading, hormone receptors and tumor cell kinetics are predictors of relapse in node-negative breast cancer to different degrees. RFS is mainly dependent on cell proliferation kinetics, represented in this study by LI. This parameter should contribute to the definition of high risk patients as a first step before the administration of medical treatment in a prospective study.

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