Prognostic significance of biological markers in node-negative breast cancer patients: a prospective study

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Report

Prognostic significance of biologic markers in node-negative breast cancer patients: a prospective study

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Summary

It is generally thought that future advances in the treatment and cure of breast cancer patients will be made possible through a deeper understanding of tumor biology and an improved capability to define the prognosis of each single patient. This will lead to the formulation of new, more selective, and patient-tailored therapies. It is therefore important, when studying potential prognostic factors, to follow methodologic requirements and guidelines which involve the carrying out of prospective studies as confirmatory steps. Repeatedly or recently investigated prognostic markers (tumor size, menopausal status, ER, PgR, ^{3}H thymidine labeling index, c-erbB-2 and p27 expression) were evaluated on a series of 286 prospectively recruited node negative breast cancer patients who underwent loco-regional treatment alone and were closely followed. The individual and relative prognostic contribution of each variable with respect to other factors, as well as their ability to identify node negative patients at risk, were assessed by univariate and multivariate analysis. At a five-year follow-up, only tumor size ($p = 0.021$) and TLI ($p = 0.016$) individually proved to be significant prognostic indicators of relapse-free survival. Conversely, p27 expression was not related to RFS and c-erbB-2 expression appeared to have only a short-term effect on patient prognosis. TLI and tumor size, tested in multivariate analysis along with ER and menopausal status, maintained their independent prognostic relevance. The study, performed on a large series of node-negative patients given loco-regional treatment alone, for the first time prospectively recruited, showed the prognostic relevance of TLI and its independence from other clinico-pathologic and biologic factors over a five-year period.

Introduction

Public awareness and widespread information campaigns about cancer prevention programs and mammographic screenings have led, in the last decade, to an earlier detection of breast cancer. The proportion of women with newly-diagnosed small and node-negative disease is therefore increasing and represents a new reality to be approached.

Consolidated clinical experience has demonstrated that about a quarter of node-negative breast cancer patients are destined to relapse and die from the disease after loco-regional treatment alone. Recent studies have also shown that adjuvant therapy improves the clinical outcome of node-negative patients [1–4], but the benefits and risks of therapy must be weighted in terms of both toxicity and cost. The clinical and biologic heterogeneity of breast cancer has led most clinicians to believe that the risk-to-benefit ratio does not favour treatment of all node-negative tumors. Consequently, an accurate prediction of the course of disease in individual patients is needed in order to identify those at risk who are candidates for adjuvant systemic treatment. It is also important for evaluating whether the patient could benefit from a particular type of therapy or should be spared the toxicity of ineffective treatment.

Over the past 20 years, new technologies have led to the proposal of an ever increasing number of potential prognostic factors for breast cancer patients.

As a result, clinicians are often faced with difficulties when trying to integrate the new and conventional prognostic factors in treatment decision-making. Furthermore, most studies have examined new biologic variables individually and almost always by retrospective analysis.

Current information in literature highlights the complexity of biologic processes underlying tumor transformation and progression such as gene alterations, cell differentiation and proliferation, apoptosis and invasiveness [5–18]. Moreover, much is now known of the multiplicity of cellular, biochemical and genetic markers representative of different biologic aspects. The large body of information derived from retrospective studies enabled us to select the markers with sufficient proof of prognostic power to be validated in a prospective study.

The aim of the present work was to assess the prognostic relevance of some biologic variables considered individually and their relative contribution with respect to pathologic factors in node negative breast cancer patients. Biologic variables repeatedly or recently investigated were determined in parallel on individual tumors in order to define their relative prognostic contribution and their ability to identify node-negative patients at risk.

Patients and methods

The clinic-biological study was conducted on a series of 286 women with node-negative breast cancer recruited by six clinical centers from 1989 to 1993. At least 10, and a median of 16, axillary lymph nodes were histologically examined. All patients had loco-regional treatment; more than 50% underwent quadrantectomy plus radiotherapy, and the remaining patients underwent mastectomy. Adjuvant systemic treatment was not given prior to relapse. The case series was consecutive on the basis of the availability of cell proliferation information. Patient distribution according to different age classes is reported in Table 1. Median age was 55 years and about 60% were postmenopausal patients, that is, more than 2 years had elapsed from spontaneous menopause or ovariectomy at the time of diagnosis.

More than two thirds of tumors were 2 cm or less in diameter and 18% were equal to or smaller than 1 cm. About 70% were ER-positive on the basis of the 10 fmol cut off, and about 55% were PgR-positive tumors on the basis of the 25 fmol cut off value.

Table 1. Clinico-pathologic and biologic characteristics of the case series

	Number of cases	Percentage
Age (years)		
$\leq$45	74	25.9
46–50	44	15.4
51–60	85	29.7
>60	83	29.0
Menopausal status		
Premenopausal	113	39.5
Postmenopausal	173	60.5
Type of surgery		
Quadrantectomy + RT	161	56.9
Mastectomy	122	43.1
Missing	3	
Histotype		
Lobular	26	9.2
Ductal	225	80.1
Others	30	10.7
Missing	5	
Tumor size		
$\leq$1.0 cm	49	18.2
1.1–2.0 cm	139	51.7
>2.0 cm	81	30.1
Missing	17	
Receptor status		
ER $\geq$ 10 fmol	198	72.0
<10 fmol	77	28.0
Missing	11	
PgR $\geq$ 25 fmol	132	54.5
<25 fmol	110	45.5
Missing	44	
TLI		
< 3.1%	143	50.0
$\geq$ 3.1%	143	50.0
c-erbB-2		
Negative	131	57.2
Positive (any % of positive cells)	98	42.8
Missing	57	
p27		
Negative	71	28.0
Positive (any % of positive cells)	183	72.0
Missing	32	

Median TLI value was 3.1% which is in agreement with the value reported in larger series [19–23]. Only membrane staining was considered in scoring c-erbB-2 positive cells; cytoplasmic staining was ignored as aspecific immunoreaction. Clear p27 nuclear staining was defined as positive. Positivity (any percentage of positive cells) for c-erbB-2 and p27 was observed in about 40% and 70% of tumors, respectively. Estrogen v (ER) and progesterone (PgR) receptors were determined in the different centers, ^{3}H-thymidine labeling index (TLI) in only two centers (Forlì and Florence), and c-erbB-2 and p27 expression was evaluated in the Forlì laboratory.

Postoperative follow-up was carried out in the outpatient clinic of all centers. All patients had a clinical and instrumental check-up at 3-month intervals for the first 2 years, every 6-months during the 3rd, 4th and 5th years, and subsequently once a year up to the 10th year. Median follow-up was 74 months (range 4–111 months). Seventeen patients were lost to follow-up (6%), of whom 11 after at least 40 months.

In vitro *determinations*

Immediately after surgery, part of the tumor material was incubated with ^{3}H-thymidine and then processed for conventional histologic procedures for the determination of TLI, c-erbB-2 and p27 expression. The rest of the tumor material was frozen in liquid nitrogen and stored at −80°C for ER and PgR determination.

TLI

Fresh tumor samples were incubated in culture medium containing ^{3}H-thymidine for 1 h at 37°C and fixed in formalin. The recent availability of a commercial kit (Euroframe, Asti, Italy) enabled all the participating centers to perform this first step of *in vitro* ^{3}H-thymidine labeling in their own laboratory. Samples from all patients were then sent to the two referee centers (Forlì and Florence) for autoradiographic procedures and TLI determination. Histologic sections were dipped in a photographic emulsion (Ilford K5, Ilford Photographics, London, UK) and exposed in the dark for 3 days at 4°C. Autoradiograms were developed in Ilford Phenprint for 6 min at 19°C and fixed in Hypam compound for 10 min. Samples were stained with hematoxylin and eosin at 4°C. When the specimen was small enough to allow the radioactive precursor to penetrate completely, labeled cells were counted throughout the whole section; if not, counting was limited to the periphery of the section (up to 80 μm in depth). TLI, expressed as the ratio between thymidine-labeled cells and the total number of tumor cells, was determined independently by two observers, with 2,000–5,000 cells scored from different fragments of the same tumor. Quality control procedures were periodically repeated in the context of a National Quality Control Program promoted by the Italian Society of Basic and Applied Cell Kinetics (SICCAB) [24].

Steroid receptor content

ER and PgR were assayed by the dextran-coated charcoal method according to the European Organization for Research and Treatment on Cancer [25]. Quality control procedures for hormone receptor dosage were coordinated by the Italian ad hoc committee. Quantitative biochemical analysis was adopted to allow the use of different cut off values and to identify different steroid receptor content subgroups for future basic and clinical analyses.

p27 and c-erbB-2 expression

Tumor samples were fixed in 10% formalin. 3 μm sections from paraffin-embedded blocks were deparaffinized with xylene, rehydrated, and endogenous peroxidase activity blocked. p27 antigen retrieval was performed by means of microwaving at 750 W in 10 mM citrate buffer (pH 6.0) for 15 min followed by cooling at room temperature for 20 min. The sections were then treated for non-specific binding with 3% bovine serum albumin in PBS for 20 min. After this they were incubated for 1 h at room temperature with p27 monoclonal antibody clone 57 (Transduction Laboratories, Lexington, KY) diluted 1:300 in PBS, for 15 min in biotynilated anti-mouse secondary antibody, then rinsed and incubated with avidin–biotin conjugate (Dako, LSAB+kit). After washing in PBS, the peroxidase reaction was developed to a brown stain by 0.05% diaminobenzidine, which was enhanced with 0.07% imidazole and hydrogen peroxide. Cell nuclei were counterstained blue by Mayer's haemalum and the sections were mounted in Faramount (Dako).

For the determination of c-erbB-2 expression, the sections were incubated for 1 h at room temperature with the monoclonal antibody CB11, which recognizes the internal domain of the c-erbB-2 protein (HER-2/neu) (Biogenex, San Ramon, CA), diluted 1:50 in antibody diluent with Background Reducing Components (DAKO Corporation, Carpinteria, CA). At this concentration c-erbB-2 overexpressing tumor

Table 2. Relationship between biologic variables and clinico-pathologic factors

	TLI		c-erbB-2		p27	
	Median value % (range)	p	Median value of positive cells % (range)	p	Median value of positive cells % (range)	p
Age						
≤ 50 years	4.0 (0.01–16.8)	0.03	40 (5–90)	0.83	18.6 (1–93)	0.95
> 50 years	2.7 (0.01–17.0)		50 (5–100)		18.2 (1–100)	
Menopausal status						
Premenopausal	3.5 (0.01–15.0)	0.15	60 (5–90)	0.50	18.0 (1–93)	0.93
Postmenopausal	2.8 (0.01–17.0)		40 (5–100)		18.6 (1–100)	
Histotype						
Lobular	3.9 (0.2–15.0)	0.04	20 (5–50)	0.09	17.5 (1–97)	0.94
Ductal	2.8 (0.01–17.0)		50 (5–100)		18.6 (1–100)	
Others	4.4 (0.01–16.8)		62 (5–90)		12.6 (1–98)	
Tumor size						
≤ 1.0 cm	2.8 (0.2–11.0)	0.02	40 (5–90)	0.21	31.7 (1–100)	0.09
1.1–2.0 cm	3.2 (0.01–16.8)		35 (5–100)		13.9 (1–95)	
> 2.0 cm	4.2 (0.2–17.0)		70 (5–100)		19.4 (1–97)	
ER						
≥ 10 fmol	2.8 (0.01–15.0)	0.01	35 (5–100)	0.16	19.6 (1–100)	0.05
<10 fmol	4.3 (0.1–17.0)		65 (5–100)		8.4 (1–94)	
PgR						
≥ 25 fmol	2.8 (0.01–15.0)	0.04	35 (5–90)	0.38	21.3 (1–98)	0.08
<25 fmol	4.0 (0.01–17.0)		55 (5–100)		13.1 (1–100)	

cells showed a strong and focalized membrane staining.

At least 20 high-power fields were scored by two independent observers for c-erbB-2 (FDP and AMG) and p27 (FDP and PB). Immunoreactivity was expressed as the ratio between the percentage of stained cells and the total number of tumor cells, or the entire area of invasive neoplastic tissue for p27 and c-erbB-2, respectively.

Statistical methods

The relationship between TLI, c-erbB-2, p27 and clinico-pathologic or biologic factors was analysed using a non-parametric ranking statistic (Median test), and Spearman's correlation coefficient was used to investigate the relationship between the different biomarkers considered as continuous variables in individual tumors.

Relapse-free survival was calculated as the period from surgery until the date of the first documented evidence of new disease manifestation in loco-regional or distant sites, or in the contralateral breast. Owing to the difficulty of distinguishing between a second breast carcinoma and contralateral recurrence, the latter lesion was considered as an event. In the case of a second primary cancer in a non-breast site, relapse-free follow-up data were censored at the time of the diagnosis of the second malignancy. All new disease manifestations were assessed by clinical, radiologic and, when feasible, histologic examination of the site of relapse. Univariate analysis was performed tracing Kaplan-Meier survival curves, and comparison of survival curves was based on the log-rank test [26].

The role of each of the putative prognostic variables (univariate analysis) and their joint effect (multivariate analysis) was evaluated using Cox proportional hazard models [27]. The analysis of the plot of ln-ln($S(t)$), (where $S(t)$ is the Kaplan-Meier estimate of the relapse-free survival curves), against the logarithm of the time for each level of the factor studied, suggested that the assumption of proportional hazards was

Table 3. Correlation between biologic variables

	PgR		TLI		c-erbB-2		p27	
	r_S	p	r_S	p	r_S	p	r_S	p
ER	0.444	<0.001	−0.197	0.001	−0.180	0.008	0.128	0.046
PgR			−0.104	0.108	−0.269	<0.001	0.127	0.063
TLI					0.195	0.003	0.046	0.463
c-erbB-2							−0.042	0.535

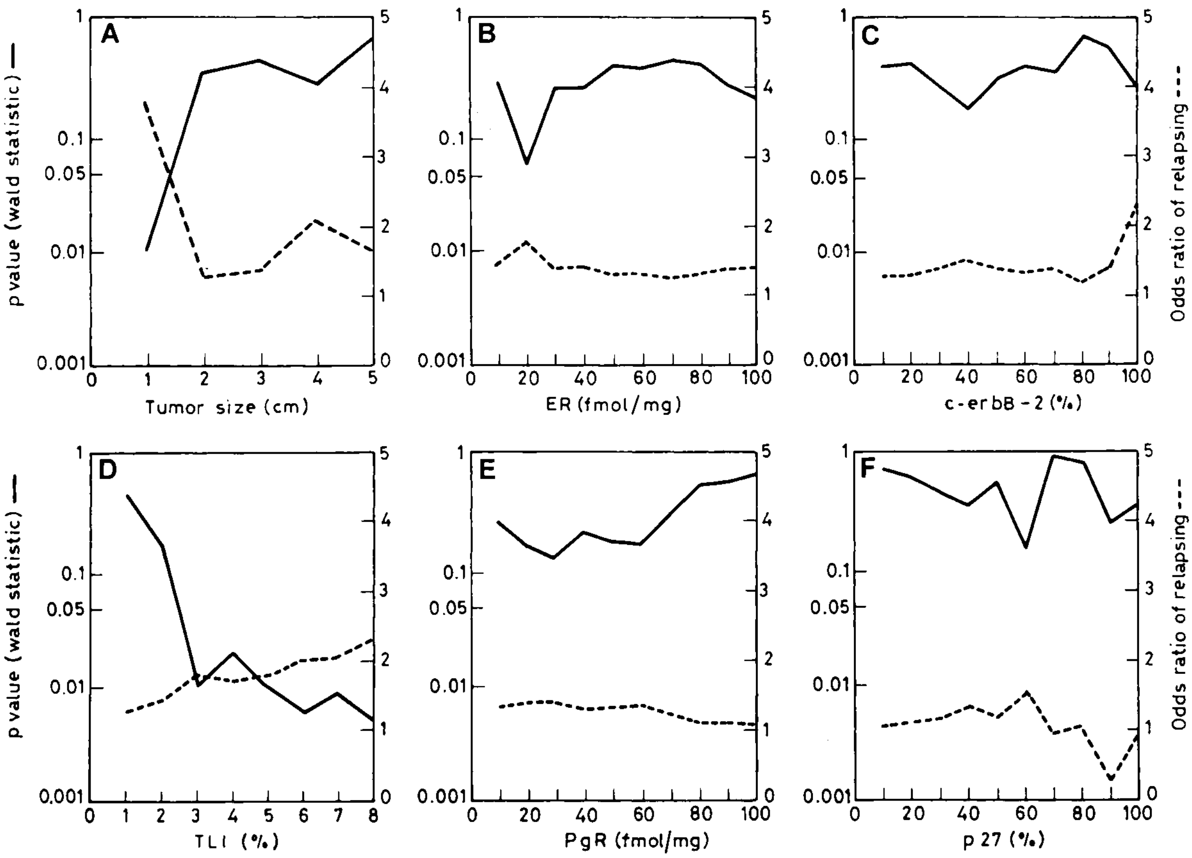

Figure 1. Cut off values for predicting 5-year relapse-free survival plotted against the relative p values (———) and Odds ratio of relapse (- - - - - - - -) (A: tumor size; B: ER; C: c-erbB-2; D: TLI; E: PgR; F: p27).

generally correct [28]. In this model, the exponential of each of the regression coefficients (β) is the odds ratio (OR), which is assumed to be constant in time. In univariate and multivariate analyses, the best putative prognosis categories were used as references. The null hypothesis $\beta_i = 0$ was tested by the Wald statistic. As the prognostic variables were categorised, one or more dummies were built for each of them.

The final model for multivariate analysis was obtained using a backward stepwise procedure. Variables that did not contribute significantly to the multivariate Cox model ($p > 0.05$) were eliminated. A forward stepwise procedure was also performed, obtaining the same results as those of the backward procedure.

To evaluate the prognostic role of the different parameters analysed in the study we tested different cut off values ranging from the minimum to the maximum value observed in our case series. Each dichotomy was used as an independent variable in a Cox regression model to predict recurrence. Each model

Table 4. Five-year relapse-free survival as a function of clinico-pathologic-biologic variables

	Number of cases	RFS (%)	95% CI	Logrank	p
Menopausal status					
Premenopausal	113	76	68–84		
Postmenopausal	173	79	72–85	0.01	0.935
Tumor size					
≤ 1.0 cm	49	94	87–100		
1.1–2.0 cm	139	75	67–82		
> 2.0 cm	81	73	63–83	7.75	0.021
TLI					
<3.1%	143	83	76–89		
$\geq 3.1\%$	143	72	65–80	5.83	0.016
ER					
≥ 10 fmol	198	76	70–82		
<10 fmol	77	79	70–88	1.21	0.271
PgR					
≥ 25 fmol	132	71	63–79		
<25 fmol	110	81	73–88	1.33	0.250
c-erbB-2					
<40%	175	80	74–86		
$\geq 40\%$	54	70	57–82	1.73	0.188
p27					
$\leq 60\%$	218	80	75–86		
$> 60\%$	36	62	45–80	2.13	0.140

was evaluated with the Wald statistic and the one with the smallest p value was identified. All p values were based on two-sided testing and statistical analyses were carried out with SAS Statistical software [29].

Results

The analysis of the relationship between biologic and pathologic variables (Table 2) showed a significantly higher proliferative activity in younger patients and a suggestive, but not statistically significant, higher TLI in pre- rather than in postmenopausal women. Moreover, TLI median value increased as a function of tumor size and was significantly higher in ER or PgR negative tumors than in steroid receptor positive tumors.

c-erbB-2 immunoreactivity was observed in about 40% of tumors and the percentage of immunoreacting cells ranged from 5% to 100%. Neither the percentage of c-erbB-2 positive tumors nor the percentage of positive cells were related to patient age or menopausal status, whereas a higher, albeit not significantly different, percentage of positive cells, was observed in larger or steroid receptor negative tumors than in smaller or steroid receptor positive tumors. Moreover, a lower percentage of positive cells was observed in lobular histotype than in ductal invasive histology. p27 positivity was observed in more than 70% of tumors and the percentage of positive cells ranged from 1% to 100%. A higher, but not significantly different ($p = 0.09$) frequency of immunoreacting cells was observed in tumors ≤ 1 cm. The percentage of positive cells was also higher in estrogen receptor ($p = 0.05$) or progesterone receptor positive ($p = 0.08$) tumors than in steroid receptor negative tumors.

The analysis of different markers analysed as continuous variables (Table 3) showed a significantly direct relation between c-erbB-2 and TLI, and an inverse

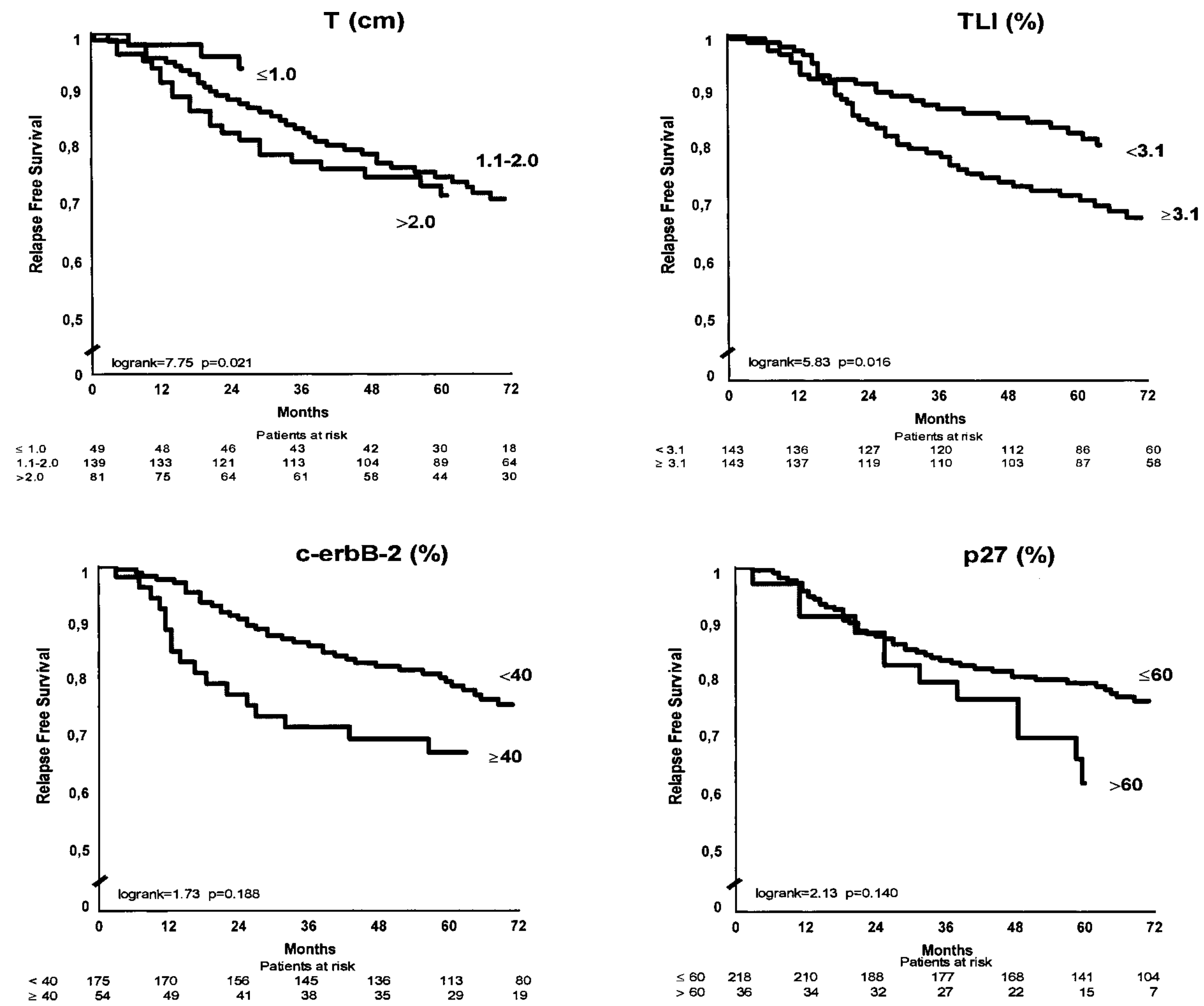

Figure 2. Relapse-free survival curves as a function of different prognostic variables.

relation between c-erbB-2 and ER and PgR. Moreover, p27 expression was significantly directly related to steroid receptor status but not to c-erbB-2 expression or proliferative activity. However, even when significant associations were observed, the correlation coefficients were very poor.

For the prognosis evaluation, tumor size, TLI, c-erbB-2 and p27 expression, and ER and PgR content were analysed as continuous or dichotomous variables. When considered as a continuous variable, pathologic tumor size showed the unique discriminant cut off value of 1 cm as predictor of risk of relapse at 5 years. TLI was able to identify subgroups of patients with a significantly different probability of relapse-free survival starting from the 3.0% of thymidine labeled cells. The odds ratio progressively increased as TLI value increased. Conversely, no value of c-erbB-2 or p27 expression and ER or PgR content reached statistical significance as predictors of relapse (Figure 1).

In the present case series of node negative patients, 5-year relapse-free survival was 77% (95% CI 73–82%), in agreement with the results reported in most clinical studies. When biologic markers were analysed as dichotomous variables using conventional cut off values or the most discriminant values detected by the previous analyses on continuous variables, a significantly lower relapse-free survival at 5 years was observed for patients with tumors larger than 1 cm or rapidly proliferating tumors than in patients with smaller ($p = 0.021$) or slowly proliferating tumors ($p = 0.016$). Conversely, 5-year relapse-free survival was not related to c-erbB-2 or p27 expression when median values, other values or negativity versus

Table 5. Results from backward application of Cox model (260 patients, 68 events)

Step	Factor	Odds ratio	95% CI	*p*
Relapse free survival				
1	Tumor size (> 1 cm vs ≤ 1 cm)	3.34	1.21–9.18	0.019
2	TLI (≥ 3.1% vs <3.1%)	1.78	1.07–2.95	0.027
Factors not entered				
ER status (≥ 10 fmol vs <10 fmol)		1.44	0.82–2.53	0.204
Menopausal status (post vs pre)		1.05	0.64–1.71	0.847

positivity were considered as criteria. Similarly, the conventional ER and PgR cut off values, as well as any other value, did not identify subgroups of patients at different probabilities of 5-year relapse-free survival (Table 4).

The profile of relapse-free survival curves over time (Figure 2) showed significantly distinct curves for patients with small and large tumors. Similarly, significantly different relapse curves were observed for patients with slowly or rapidly proliferating tumors. In particular, diversification started from the first year and progressively increased up to the 5th year of follow-up.

Conversely, relapse-free survival analysed as a function of c-erbB-2 expression showed a higher risk in patients with tumors with a high rather than low c-erbB-2 expression. The difference increased starting from the 1st year up to the 3rd year and then tended to diminish progressively. The curves appeared to be getting closer together at the 5th year follow-up. Overall, the relapse-free survival risk was not significantly different for the two biologic subgroups. The relapse-free survival curves for patients with weakly or highly p27-expressing tumors were not significantly different and were superimposible, at least up to the 3rd year.

The variables which individually proved to be prognostic indicators, that is tumor size and TLI, in addition to ER status and menopausal status which have long been considered to be important indicators of the natural history of breast cancer, were tested in multivariate analysis. Only tumor size and, among the biologic variables investigated, cell proliferation, maintained their independent prognostic relevance on relapse-free survival (Table 5). Relapse-free survival curves for the four subgroups defined by the independent prognostic variables identified by multivariate analysis are shown in Figure 3. For patients with tumors ≤ 1 cm, 5-year relapse-free survival was 94.5% (95% CI 89.1–100.0) when TLI was lower, and 89.9% (95% CI 80.7–100.0) when TLI was higher than ≥ 3.1%. In patients with tumors > 1 cm, 5-year relapse-free survival was 81.8% (95% CI 74.5–89.0) when TLI was low and 68.6% (95% CI 61.0–77.1) when TLI was high.

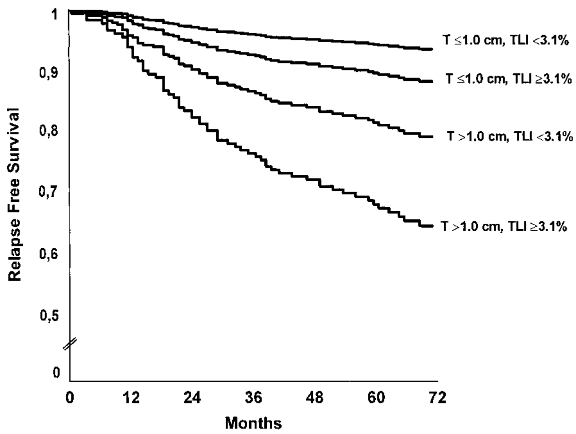

Figure 3. Relapse-free survival from the multivariate analysis.

Discussion

Our study, performed on a large series of node-negative breast cancer patients given loco-regional treatment alone, showed, for the first time in a prospectively recruited series, the prognostic relevance of TLI and its independence from other clinico-pathologic and biologic factors over a 5-year follow-up period. These results confirm that tumor proliferative activity, evaluated as TLI, is a strong and reproducible indicator of tumor aggressiveness, as already proven by pilot and confirmatory retrospective studies on very large series of cases and with longer follow-up periods [30–41].

The reproducibility of TLI as an indicator of relapse-free survival and survival is favoured by the unequivocal autoradiographic image of labeled cells as compared to the modulation of immunohistochemical images, and guaranteed by an ongoing National Quality Control Program, which was activated in Italy almost 10 years ago [24]. Therefore, TLI represents an important variable for identifying patients with negative lymph-node tumors at risk who are thus candidates for adjuvant systemic therapy. Pathologic tumor size was also an independent prognostic indicator with a 94% disease-free probability at 5 years for patients with very small tumors. In contrast, in the present series of patients, hormonal receptor status did not affect the 5-year incidence of new disease manifestation. Moreover, the four subgroups defined according to TLI and pathologic tumor size, which were the only two independent prognostic variables identified by multivariate analysis, showed a significantly different risk of relapse. The risk was lowest for the subgroups of patients with the two favourable prognostic variables (about 5%) and more than six times higher for patients with the two unfavourable prognostic variables.

The fraction of c-erbB-2 expressing cells was not related to either patient age or menopausal status. In contrast, it was noticeably higher in ductal than in lobular histotype, in agreement with other results [42], and, as already reported, progressively increased as tumor size increased [43–46] and steroid receptor content decreased [42, 43, 47–51]. Moreover, we confirmed the relation between c-erbB-2 and cell proliferation. As far as we know, the only two papers which to date have investigated the relationship between c-erbB-2 expression and TLI in breast cancer found a significant association between high TLI and positive staining in both *in situ* [52] and invasive carcinoma [53].

The results on the prognostic value of c-erbB-2 are still somewhat contradictory, with the majority of studies reporting no prognostic relevance in node-negative breast cancers [42, 54–57]. The controversial results have been tentatively ascribed to the different follow-up periods considered in the studies [42]. The results from the present work appear to reinforce this hypothesis, identifying, for the first time experimentally, c-erbB-2 expression as a short-term prognostic indicator. This finding further confirms the lack of long-term prognostic relevance of c-erbB-2, which, conversely, remains a potential predictor of response to hormonal therapy and to different antitumor drugs [58–63].

Similarly, the cell cycle inhibitor p27 was more frequently expressed in lobular than in ductal histotypes, but the difference did not reach statistical significance, probably due to unbalanced subgroups, and was inversely related to tumor size and directly related to steroid receptor content. In our case series of node-negative breast cancer patients treated with loco-regional therapy alone, p27 expression was not related to disease-free survival. This result cannot easily be compared with the findings obtained in other studies on large series of breast cancer patients. No information on the frequency of positive or negative p27 tumors is reported and the generally used semi-quantitative analysis is not a guarantee of equivalent evaluation criteria, even though similar cut off values of p27 positive cells were adopted in most of the studies for follow-up analysis. A prognostic relevance of p27 expression was observed in studies including both node-positive and node-negative patients considered as one group and treated with different types of chemotherapy or hormone therapy [64], or for whom treatment modalities are not specified [65–67]. The results on node negative patients are less consistent. In fact, a relation between the cell cycle inhibitor and clinical outcome was reported by Porter [67] and Wu [66] but not by Tan [64], who performed, as we did, a quantitative analysis of the percentage of p27 positive cells. However, it must also be pointed out that in all these studies, node-negative patients were treated with different types of systemic therapy, whereas our analysis was performed for the first time, as far as we know, on node-negative patients who underwent only loco-regional therapy. Our results are in agreement with those from a similar study recently published by Reed et al. [68].

In conclusion, TLI remains, in our experience, a variable of important clinical relevance by intrinsically containing both prognostic and predictive information on response to chemo- and hormone therapy [69–73]. Therefore, once node negative patients at risk have been identified on the basis of tumor size and cell proliferation, TLI, together with steroid receptors and c-erbB-2 expression, could open a new era of individually-tailored therapy for patients.

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