Modulation of Biomarkers in Minimal Breast Carcinoma

A Model for Human Breast Carcinoma Progression

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BACKGROUND. The widespread use of diagnostic breast imaging has yielded an increase in the detection of in situ, microinvasive, and small invasive carcinomas and has provided opportunities to study the earliest stages of breast carcinoma development. The authors of this report analyzed the pathobiologic features of 577 minimal breast carcinomas (MBCs), including in situ carcinomas and invasive carcinomas ≤1 cm, according to the definition given by Hartmann in *Cancer* (1984;53:681–4).

METHODS. Estrogen and progesterone receptors (ER and PR), proliferation index (PI), and p53 and *neu* expression were studied by immunohistochemical technique and measured by quantitative image analysis in 99 pure in situ carcinomas (ISCp); in 105 mixed invasive/in situ carcinomas, with a separate analysis of in situ (ISCm) and invasive (ICm) components; and in 373 invasive carcinomas ≤1 cm (IC). Follow-up data were available for 164 invasive carcinomas.

RESULTS. A progressive increase in the levels of hormone steroid receptors, from the lowest in ISCm to the highest in IC, was observed (ER, $P < 0.001$; PR, $P = 0.005$). Levels of PI and p53 expression were higher in ISCm than in the other categories (PI, $P = 0.007$; p53, $P = 0.046$). Overexpression of *neu* was greater in ICm than in IC ($P = 0.013$). Younger women (≤40 years) with invasive carcinoma had worse biologic profiles, with lower ER ($P < 0.001$) and higher PI ($P = 0.021$), *neu* ($P = 0.008$), and p53 ($P = 0.040$). It was demonstrated clinically that PI and *neu* were the biologic markers with the highest predictive prognostic values in univariate analysis (PI for recurrence, $P < 0.015$; *neu* for recurrence and overall survival, $P < 0.001$ and $P < 0.007$, respectively) and in multivariate analysis (*neu* for recurrence and overall survival, $P < 0.007$ and $P < 0.017$, respectively).

CONCLUSIONS. Biologic phenotypes of MBC can be interpreted as reflecting a dimension of neoplastic progression capacity that is independent of tumor size. This study suggests that biologic markers can be integrated with traditional pathologic indicators for accurate staging of patients. ***Cancer* 1998;83:89–97.**
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KEYWORDS: minimal breast carcinoma, estrogen receptor, progesterone receptor, proliferation index, *neu,* p53, menopausal status, age groups, relapse-free interval, overall survival.

The definition of minimal breast carcinoma (MBC), as introduced by Gallager and Martin in 1971, encompasses in situ carcinoma (ISC) of any dimension and invasive carcinoma (IC) up to 0.5 cm in greatest dimension.[3] Subsequently, Hartmann (1984) included in this group ISC and IC equal to or less than 1 cm in greatest dimension.[1]

Since mammography, combined with stereotaxic fine-needle technique, has come to be used more widely, the observed spectrum

of breast pathology has changed, with an increased detection rate of in situ, microinvasive, and invasive carcinomas with size less or equal than 1 cm, providing the opportunity to study both premalignant as well as the earliest stages of breast carcinoma development.[3–5] In recent years, many efforts have been made to gain a deeper insight into the natural history of these tumors and their prognostic factors in order to define a tailored management.[6,7]

In this study, we analyzed the pathologic and biologic features of 577 MBC: 99 ISCs and 478 ICs ≤1 cm. In 105 cases, we analyzed in situ components of ICs, comparing them with the infiltrating component of the same tumors.

These cases were selected from among more than 2,400 breast carcinomas, which were collected in the same period, whose biopathologic characterization was examined. To verify the different weight of biologic markers in neoplastic progression and their prognostic significance in invasive tumors, a clinical study was performed on cases with available follow-up data.

PATIENTS AND METHODS

Five-hundred and seventy-seven cases of MBC, 478 ICs with size ≤1 cm (pt1a-b) and 99 ISCs (from 74 patients), were retrieved from a consecutive series of breast carcinomas collected in our Pathology Department from 1985. On these cases, an immunohistochemical (ICA) study for estrogen and progesterone receptors (ER and PR), proliferation index (PI), and *neu* and p53 over-expression, was performed. Clinicopathologic features of 577 MBCs are reported in Table 1. The size of each invasive tumor was determined by direct measurement of histologic section. Patients' menopausal status was recorded at diagnosis. Women were classified as menopausal when amenorreha had persisted for at least 2 years. The median age of patients with ISC was 56 years (range, 25–89 years); 41.9% were premenopausal. The histologic diagnosis was ductal carcinoma in situ (DCIS), 84 cases; lobular carcinoma in situ (LCIS), 15 cases. DCIS was subclassified according to morphological growth pattern into cribriform (16 cases); noncomedo, which are solid, micropapillary, papillary types (40 cases); and comedo (28 cases). DCIS cribriform and DCIS noncomedo differ in architectural growth pattern: Cribriform type is characterized by spaces of similar size, whereas noncomedo type has a micropapillary, papillary, or solid growth pattern.

The median age of patients with IC was 59 years (range, 32–89 years), 39% were premenopausal, and 78.1% were lymph node negative (N^-). The rate of nodal metastases was 1.4% for T1a category, 20.5% for T1b. Tumor size was ≤0.5 cm in 15.5% and 0.6–1.0 cm in 84.5% of patients.

TABLE 1
Clinicopathologic Features of 577 Minimal Breast Carcinomas

Variable	Values
In situ carcinomas (99 cases)	
Age at diagnosis	
Median	56.9
Menopausal status	
Pre	41.9%
Cytologic diagnosis (55 cases)	
Positive	43.6%
Suspect	50.9%
Inadequate	5.5%
Histologic type	
DCIS cribriform	16.2%
DCIS noncomedo	40.3%
DCIS comedo	28.3%
LCIS	15.2%
Invasive carcinomas pt1 a-b (478 cases)	
Age at diagnosis	
Median	59
Menopausal status	
Pre	39%
Cytologic diagnosis (254 cases)[a]	
Positive	61.4%
Suspect	31.4%
Negative	3.5%
Inadequate	3.5%
Lymph node status	
Negative	78.1%
Histologic type	
Ductal	69.3%
Lobular	15.6%
Special types	15.1%

DCIS: ductal carcinoma in situ; LCIS: lobular carcinoma in situ.
[a] Eighty-two cases with stereotaxic fine-needle aspiration.

The histologic type was distributed as follows: ductal, 69.3%; lobular, 15.6%; special types, 15.1% (tubular, 9.4%; papillary, 2.1%; cribriform and mucinous, 1.5%; medullary, 0.6%). According to the International Union Against Cancer/TNM staging system,[8] patients were classified as follows: 77.8% Stage I, 20.6% Stage IIa, and 1.6% Stage IIIa.

In ICs, the presence of an adjacent in situ component was recorded only when it was extensive/predominant. According to its relative amount, in situ component was classified as extensive/predominant when it comprised greater than 50% of the tumor area. The topographical distribution was also evaluated considering in situ component within (predominant) or adjacent (extensive) to invasive tumor, in accordance with the World Health Organization histologic classification of breast tumors, the recommendations for the reporting of breast carcinoma by the Associa-

tion of Directors of Anatomic and Surgical Pathology and other authors.[9–12] Cases with 25–50% of in situ component were not recorded for our analysis. One hundred and five ICs (21.9%) showed an extensive/predominant in situ component (mixed IC/ISC), classified as cribriform (12.4% of cases), noncomedo (45.7% of cases), comedo (38.1% of cases), and lobular (3.8% of cases). Biologic profile of IC was also evaluated for three age groups: ≤40 years (24 patients), 41–55 years (162 patients), and >55 years (292 patients) at diagnosis. Follow-up data were available for 164 patients with infiltrating carcinoma who had been operated between January 1985 and December 1991 (median follow-up, 54.9 months). The median age was 54 years (range, 32–85 years), 37.2% were premenopausal, and 74% were N^-.

The ICA assays for IC were performed on paraffin-embedded sections in 40.2% of cases and on cryostatic sections on the other 59.8%. On in situ lesions, the biologic parameters were always investigated on paraffin-embedded sections.

Immunohistochemistry

Hormonal receptors were evaluated with H222 (ER-ICA Abbott; Abbott Laboratories, Chicago, IL) and KD 68 (PR-ICA Abbott). Staining procedures of cryostatic sections were performed according to the instructions included in the ER-ICA and PR-ICA kits. The fixed and paraffin-embedded sections were stained by using a streptavidine biotin-peroxidase method (Biogenex, San Ramon, CA). Pronase predigestion was performed on sections stained with H222.

Proliferation index on cryostatic sections was assessed with Ki-67 (Dako, Glostrup, Denmark) at 1:250 dilution and, on paraffin-embedded specimens, with MIB1 (Dianova Immunotech, Marseille, France) at 1:250 dilution. Neu protein was shown with Ab-1 (Triton Diagnostics, Alameda, CA) at a dilution of 1:100. P53 protein over-expression was examined with D07 (Dako) at a dilution of 1:25. Ki-67, MIB1, and D07 antibodies were detected by using the avidin-biotin-peroxidase complex (ABC) method (Vectastain ABC Kit; Vector Laboratories, Burlingame, CA). Microwave pretreatment was performed on sections stained with MIB1 and D07.

In all ICA assays, 3,3′-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO) was used for the chromogen, and ethyl green stain was used for nuclear counterstaining, according to the procedure described in the kit (Cell Analysis System, Elmhurst, IL).

Quantitation of Immunohistochemical Staining

The evaluation of ER, PR, PI (Ki-67/MIB1), p53, and *neu* was quantified with an image-analysis computerized system (CAS 200; Becton-Dickinson, San Jose, CA).[13,14] The percentage of positive-stained nuclei (PI, ER, PR, and p53) and cell plasma membrane (*neu*) is the proportion of the positively stained area on the total area considered. The use of an interactive window command allows to the operator to select the fields containing carcinoma cells and excludes any other area with nonneoplastic components. For each tumor section, at least 25 microscopic fields of invasive carcinoma (×40 objective) and 40,000 μm^2 of nuclear area, selected at random, were measured. At ≥15 fields of vision, the positive nuclear area (PNA) has a low standard deviation (SD) and a stable coefficient of variation. For the PI, at least 2,000 nuclei were measured. For quantitative evaluation of biologic markers on ICs, a lesion field method was used, with the aim of making the lesion histologically homogeneous: Each section was investigated (×40 objective), evaluating all neoplastic fields present. When mixed tumors were investigated, the single histologic type was evaluated separately. This method allows evaluation of the biologic profile of each single histologic type.[15]

The 10% of positive area was used as a cut-off value for ER, PR, and *neu:* The 5% was used for p53; the 13% of PI was used to divide cases with low and high proliferative activity. The cut-off values were selected according to our and others' experience with evaluating clinical behavior.[16–19]

Data Analysis

Statistical analysis was performed with the SPSS-X and BMDP packages (SPSS Inc., Chicago, IL) operating on an IBM S/390 computer (IBM S.E.M.E.A. S.p.a., Milan, Italy). Correlations and differencies were analyzed by using nonparametric models (chi-square, Spearman's correlation, Mann–Whitney U, and Kruskal-Wallis tests). The clinical follow-up univariate analysis for relapse-free interval (RFI) and overall survival (OS) was performed with the actuarial model and Lee-Desu statistics.[20] Logistic regression and Cox proportional hazards model were further applied to evaluate predictors of lymph node status and clinical outcome.

RESULTS

The biologic behavior of 577 MBCs was investigated by analysis of 99 ''pure'' ISCs (ISCp), 105 mixed invasive/ISCs, with a separate analysis of in situ (ISCm) and invasive component (ICm) of the same tumor, and 373 ICs without extensive/predominant in situ

TABLE 2
Biologic Phenotypes of 577 Minimal Breast Carcinomas

Biophenotype	ER > 10% (%)	PR > 10% (%)	PI > 13% (%)	*neu* > 10% (%)	p53 > 5% (%)
Pure ISC (99 cases)					
Cribriform	100	92.3	0	0	8.3
DCIS					
Noncomedo	79.5	71.1	5.3	13.2	23.5
Comedo	33.3	25.9	68	73.1	42.1
LCIS	100	46.7	0	13.3	26.7
	$P < 0.001$	$P = 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.205$
Mixed ISC (105 cases)					
Cribriform	100	100	0	0	0
DCIS					
Noncomedo	85.3	79.4	18.2	25.7	32.3
Comedo	30.3	27.3	80	51.6	72.4
LCIS	100	50	0	0	0
	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.005$	$P < 0.001$
Mixed IC (105 cases)					
Special types	86.4	85.7	5.6	15	12.5
Lobular	100	87.5	14.3	25	0
Ductal	63.9	50	37.7	38.6	38.2
	$P = 0.020$	$P = 0.004$	$P = 0.019$	$P = 0.125$	$P = 0.228$
IC (373 cases)					
Special types	95.6	75.6	6.7	9.3	16.7
Lobular	88.3	77.6	9.1	12.3	18.6
Ductal	84.3	72.2	26.4	22.8	33.3
	$P = 0.114$	$P = 0.679$	$P < 0.001$	$P = 0.043$	$P = 0.052$

ISC: in situ carcinoma; DCIS: ductal carcinoma in situ; LCIS: lobular carcinoma in situ; IC: invasive carcinoma; ER: estrogen receptor; PR: progesteron receptor; PI: proliferation index.

component (IC). The biophenotypes obtained are outlined in Table 2.

In Situ Carcinomas (Pure and Mixed)

Estrogen receptor was expressed in 100% of cribriform and 100% of lobular types both in ISCp as well as in ISCm; ER expression among the four histotypes considered was different ($P < 0.001$). Progesterone receptor had the highest value in cribriform DCIS in both groups evaluated ($P < 0.001$). The expression of PI, *neu,* and p53 was higher in comedo type in both categories, but, for p53, the difference did not reach statistical significance in ISCp ($P = 0.205$). Lobular carcinoma in situ displayed a reduced PR expression compared with ER (46.7% versus 100% in ISCp; 50% versus 100% in ISCm). Among DCIS, noncomedo types showed an intermediate biophenotype in both groups.

Invasive Carcinomas (Invasive Component/Without In Situ Component)

The expression of hormone steroid receptors was higher in lobular and special types both in ICm and IC but, in IC, failed to reach statistical significance (ER, $P = 0.114$; PR $P = 0.679$). Ductal type displayed the highest PI, *neu,* and p53 expression in both groups,

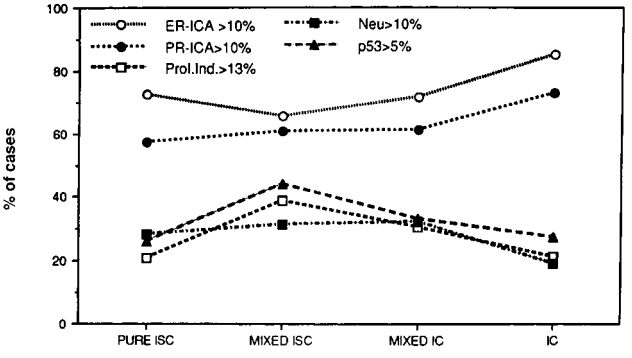

FIGURE 1. Modulation of biomarkers in minimal breast carcinoma: different distribution of estrogen and progestrone receptors (ER, PR), proliferation index (Prol Ind), *neu,* and p53 from pure in situ carcinoma (ISCp) to invasive carcinoma (IC).

with statistically significant values for PI (ICm: 37.7%, $P = 0.019$; IC: 26.4%, $P < 0.001$) and for *neu* in IC (22.8%, $P = 0.043$).

The progression of biologic marker expression (Fig. 1), from ISCp, to IC, through mixed IC/ISC, showed a progressive increase of hormone steroid receptors from ISCm, with the highest levels reached in IC (ER, $P < 0.001$; PR, $P = 0.005$). Proliferation index and p53 expression had the highest levels in ISCm

TABLE 3
Modulation of Biomarkers and Histological Types in Minimal Breast Carcinoma

Biomarker	Pure ISC (%)	Mixed ISC (%)	Mixed IC (%)	IC (%)
ER > 10%	72.6	65.4	71.8 ($P = 0.002$)	85.3
PR > 10%	57	60.5	61 ($P = 0.022$)	73
PI > 13%	21.1 ($P = 0.012$)	39	30.5	21.6
neu > 10%	28.3	31.3	32.3 ($P = 0.006$)	19.3
p53 > 5%	26.3 ($P = 0.021$)	44.3	33.3	27.7

ISC: in situ carcinoma; IC: invasive carcinoma; ER: estrogen receptor; PR: progesterone receptor; PI: proliferation index.

TABLE 4
Modulation of Biomarkers and Histological Types According to Menopause in Minimal Breast Carcinoma

Biomarker	Pure ISC (%)	Mixed ISC (%)	Mixed IC (%)	IC (%)
Premenopause				
ER > 10%	68.3	56.8	63.3	76.6
PR > 10%	50	54.1	61.7	74.1
PI > 13%	15.8 ($P = 0.001$)	52.8	39.1	24.1
neu > 10%	22.2	35.1	34.8	27.3
P53 > 5%	20.7 ($P = 0.009$)	52.9	45	31.6
Postmenopause				
ER > 10%	75.9	72.7	79.6 ($P = 0.032$)	90.2
PR > 10%	62.7	65.9	60.4	72.4
PI > 13%	25	26.8	22.4	20.3
neu > 10%	32.1	27.9	30.2 ($P = 0.011$)	15.1
P53 > 5%	29.4	36.1	24	25.6

ISC: in situ carcinoma; IC: invasive carcinoma; ER: estrogen receptor; PR: progesterone receptor; PI: proliferation index.

compared with other groups (PI, $P = 0.007$; p53, $P = 0.046$). *neu* over-expression had the highest value in ICm ($P = 0.013$).

The comparison between histopathologic categories and biologic marker expression (Table 3) showed statistically significant differences between ISCp and ISCm in PI (21.1% versus 39%; $P = 0.012$) and p53 expression (26.3% versus 44.3%; $P = 0.021$) and between ICm and IC in ER (71.8% versus 85.3%; $P = 0.002$), PR (61% versus 73%; $P = 0.022$), and *neu* expression (32.3% versus 19.3%; $P = 0.006$). According to menopausal status (Table 4), significant differences were observed: PI and p53 differed significantly between ISCp and ISCm (PI, 15.8% versus 52.8%, $P = 0.001$; p53, 20.7% versus 52.9%, $P = 0.009$) in premenopausal patients, whereas, in postmenopausal patients, differences were recorded between ICm and IC for ER and *neu* expression (ER 79.6% versus 90.2%, $P = 0.032$; *neu* 30.2% versus 15.1%, $P = 0.011$).

The distribution of 478 ICs according to age groups and biopathologic profile is summarized in Table 5. Younger patients (≤40 years), who constituted 5% of the total group, differed from others by a lower ER (40.9%; $P < 0.001$), higher PI (47.4%; $P = 0.021$), and higher *neu* and p53 (42%, $P = 0.008$; 48.3%, $P = 0.04$) expression. No significance was reached for PR and lymph node status.

TABLE 5
Biopathologic Profile in 478 Invasive Breast Carcinomas ≤1 cm According to Age Group

Biomarker	< 40 yrs (%)	41–55 yrs (%)	> 55 yrs (%)	*P*
N+	22.7	27.6	18.6	NS
ER > 10%	59.1	74.7	88.2	< 0.001
PR > 10%	71.4	70.8	70.1	NS
PI > 13%	47.4	26.1	20.6	0.021
neu > 10%	42	27.9	18	0.008
p53 > 5%	48.3	31	25.3	0.004

NS: not significant; ER: estrogen receptor; PR: progestrone receptor; PI: proliferation index.

Comparing histopathologic and biologic markers with lymph node status in 478 ICs ≤1 cm, node-negative patients (versus node-positive patients) showed a lower tumor size (7.1% versus 18.1%; $P < 0.007$): No statistically significant differences were observed for ER-positive tumors (79.3% versus 83.3%), PR-positive (67.4% versus 71.6%), low PI (70.5% versus 78.3%), low *neu* expression (72.1% versus 78.2%), and low p53 expression (71.2% versus 71%). Invasive tu-

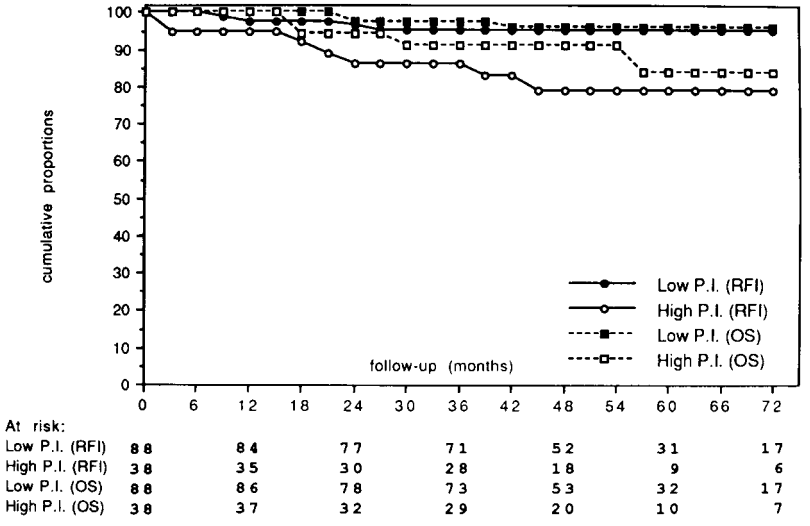

FIGURE 2. Prognostic power of proliferation index (PI) in pT1 a-b tumors: relapse-free interval (RFI), $P = 0.015$; overall survival (OS), $P = 0.132$ (Lee–Desu statistic).

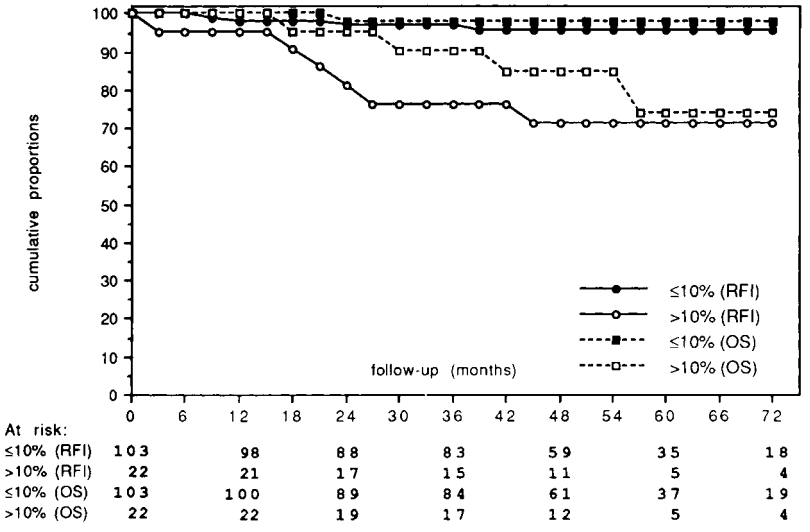

FIGURE 3. Prognostic power of *neu* assessment in pT1 a-b tumors: relapse-free interval (RFI), $P < 0.001$; overall survival (OS), $P = 0.007$ (Lee–Desu statistic).

mors, detected by stereotaxic fine needle aspiration (82 cases), were most often N^- ($P = 0.012$), ER-positive ($P < 0.001$), and had a lower PI ($P = 0.049$) compared with clinical cancers (Mann–Whitney U test). The multivariate analysis, which was performed on ICs by using a logistic regression model (using pathologic and biologic markers as covariates) with the aim of estimating the risk of axillary lymph node metastases, showed ductal histologic type [versus special types; odds ratio (OR) = 2.09; $P = 0.015$] and greatest tumor dimension >0.5 cm and ≤1 cm (versus ≤0.5 cm; OR = 3.07; $P = 0.029$) as the strongest predictors of nodal involvement.

Clinical Outcome

In 164 patients who were included in clinical study, follow-up data were available (median = 54.9 months). Seventy-eight percent and 65.1% of tumors were ER and PR positive, respectively, 30.2% had a high PI, and 17.6% and 29.6% overexpressed *neu* and p53, respectively. A univariate analysis of biologic parameters showed low PI and *neu*-negative as groups with lower probability of recurrence ($P < 0.015$ and $P < 0.001$, respectively), whereas *neu*-negative patients also had higher overall survival ($P < 0.007$; Figs. 2 and 3).

Cox proportional hazards model applied on 164 patients with IC (with lymph node status, tumor size, age at diagnosis, *neu,* and PI as covariates) highlighted lymph node status and *neu* assessment as the strongest predictors of clinical outcome. Patients with lymph node metastases had a relative risk (RR) of recurrence and death of 9.8 and 10.8, respectively; *neu*-positive tumors gave patients an RR of 5.9 for recurrence and 7.6 for death. Age at diagnosis and tumor size were not independent variables at multi-

variate analysis. Pt1a and pt1b patients did not differ significantly in clinical outcome: A greater cohort of patients and a longer follow-up period could be necessary for such evaluation.

DISCUSSION

Minimal breast carcinoma has become an important and controversial topic for breast carcinoma management, because the widespread use of mammography, combined with stereotaxic technique, has yielded an increased detection of early breast lesions.[21,22] Minimal breast carcinoma is a heterogeneous entity: A spectrum of histologic features has been recognized and correlated with quantitative biologic behavior.[19,20] Nevertheless, MBC can offer the possibility to study the progression of breast carcinoma from preinvasive (ISCs), through ICs with a predominant/extensive in situ component, to infiltrating tumors with size $\leq$1 cm. Biologic profile assessment by ICA is the optimal method to study these lesions, because the morphologic and topographic details are preserved, and the same material that is employed for routine diagnosis may be investigated. In our department, MBC represents more than 25% of breast tumors (ISC, 5%; IC $\leq$1 cm, 21.5%). This frequency is in agreement with that reported in other series.[25]

Natural History

Our study differs from others that have addressed the histopathological features of MBC, because we studied multiple biologic factors (ER, PR, PI, *neu,* and p53) that may prove to be helpful when they are used together with morphological factors.[26–28] The analysis of various biological markers by ICA yielded ''tumor biologic phenotypes'' that can be useful to better understand the phases of mammary carcinogenesis and the different risks of neoplastic progression for practical purposes.[29,30]

Our study shows a high hormone receptor content in MBC, with a progressive increase of PR from ISC to IC and of ER from ISCm to IC. Well-differentiated morphologic subtypes, such as cribriform DCIS, invasive special types, and lobular types, as expected, tend to contain higher levels of ER and PR than comedo DCIS and invasive ductal types, respectively.

Hormone receptor status is age related: premenopausal patients showed lower ER and PR values compared with postmenopausal patients.[16] This is also true in MBC, in which older patients displayed a significantly higher ER content in ICm vs IC. Our results show that markers related to biologic aggressiveness in invasive breast cancer (PI, *neu,* and p53) are observed in preinvasive steps with heterogeneous expression in different morphologically stages recognized.[15,31,32]

High proliferative activity and *neu* and p53 overexpression are detected more frequently in comedo DCIS and in invasive ductal type. Similar observations have been made in previous studies on the basis of clinicopathological data.[19,33–35] Over-expression of p53 (which is most often associated with point mutations) leads to loss of its negative growth regulation and it is related to more rapid cell proliferation, confering a more aggressive phenotype with a poor prognosis.[36]

In the current study, premenopausal patients with ISCm show a higher PI and p53 over-expression, compared to those with ISCp; no difference between ISCm/ICm and IC is observed, suggesting that p53 overexpression confers a selective growth advantage, in accordance with the high proliferative rate; this could be enhance the risk of mammary tumor progression from in situ to invasive disease.

Moreover, we have extended our analysis of MBC to age distribution: invasive breast cancer developing at young age, presents a worse biological profile (high PI, *neu,* p53 and lack of ER overexpression) with respect to other age groups, as observed in other studies about poor prognosis in younger ages.[37–40] This suggests that young women have more aggressive disease, probably due to different molecular mechanisms involved in carcinogenesis.

We also observed, as noted previously,[41] a higher percentage of *neu* protein in ISCs than in ICs, suggesting that it could represent an early event in breast carcinogenesis. Another possible explanation is that *neu* positivity is associated with persistence of the in situ stage longer than in some other carcinomas. It is noteworthy that, in postmenopausal patients, IC was less likely to express *neu* oncogene than ICm, indicating that not all in situ lesions over-expressing *neu* (comedo type) develop invasive cancers. These results suggest that some in situ tumors fails to progress to ICs during the lifetime of the patients, and some ICs may never have gone through an in situ stage. A possible reason is that some types of carcinomas develop a prominent in situ growth prior to invading (e.g., comedo, according to Barnes et al.),[42] and others do not.

Clinical History

Several studies have shown that carcinomas detected by screening programs have pathological and biologic characteristics that are suggestive of a lower malignant potential. In the present study, cases that were diagnosed through mammography combined with stereotaxic fine needle technique were more frequently node negative, ER-positive, and had a lower proliferation

rate with respect to other cases. These data confirm that nonpalpable tumors are clearly different from the clinically presenting tumors in pathologic and biologic features.[24]

In our study, clinical validation of biologic parameters performed in 164 cases suggested lymph node status and *neu* as independent prognostic factors for recurrence and death. Biologic factors integrated with traditional histopathologic parameters were able to define a subpopulation of patients with less favorable prognosis who may benefit from adjuvant treatment.

The question of axillary dissection is one of the most controversial topics, particularly in treatment of pt1a-b tumors.[43,44] In our study, size (within the range considered) and histologic type were able to predict axillary node involvement, whereas biologic markers did not. This suggests that axillary node dissection should be spared on patients with pt1a (≤5 mm) and special tumor types.

In conclusion, this study provides further evidence that, among MBC patients, a ''high-risk'' group may be distinguished by means of biologic markers (high PI and *neu* and p53 over-expression) that are easily measured today. The demonstration by our group of the different biologic markers expression in MBC raises some question about the traditional concept of in situ breast carcinoma as a midpoint in the progression from normal to invasive disease. Biologic phenotypes can be interpreted to reflect a dimension of neoplastic progression capacity independent of the small tumor sizes considered. Biologic markers can contribute to correct patient staging for more adequate treatment strategies.

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