

Hormonal receptors, cell proliferation fraction (Ki-67) and c-erbB-2 amplification in breast cancer. Relationship between differentiation degree and axillary lymph node metastases

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Summary. In view of the limitations of conventional prognostic factors such as differentiation degree, metastatic lymph nodes, hormonal receptors and others, especially when early lesions are found, additional new markers have been studied, such as gene amplification and cell proliferation index, in order to choose the appropriate treatment. Primary breast carcinoma tumors from 97 patients were examined for differentiation degree, metastatic lymph nodes, hormonal receptors, c-erbB-2 amplification and cell proliferation index (Ki-67).

A negative relationship with hormonal receptors and c-erbB-2 amplification, Ki-67 and differentiation degree was found, whereas the relationship between c-erbB-2, Ki-67 and differentiation degree was positive.

No relationship was found between these factors and metastatic lymph nodes.

The concurrence of high cell proliferation index, c-erbB-2 amplification and negative hormonal receptor presence would indicate a subpopulation with a high risk of recurrence. But a larger survival study is necessary to correlate these factors with clinical outcome.

Key words: Breast cancer, c-erbB-2, Hormonal receptors, Immunohistochemistry, Ki-67

Introduction

Breast carcinoma is the most frequent malignant tumor in women (26%). Despite hormonal and chemotherapy advances, the mortality rate has not diminished. It would be interesting, through further studies, to find some prognostic factors which could help us choose the best therapy in each case, such as the relationship

between classical prognostic factors (i.e. tumor size, differentiation degree, histological type, lymph node metastases) and new ones (i.e. cell proliferation factor and oncogene amplification (Elledge et al., 1992)). The determination of hormonal receptors (HR), cell proliferation and oncogene expression was initially performed using techniques difficult to apply in routine pathology work (biochemical determination, S phase in flow cytometry and Southern or Northern Blot, respectively). Today, such determinations can be performed using immunohistochemical techniques with monoclonal antibodies (MAb).

In recent years these immunohistochemical techniques have been used to find estrogen (ER) and progesterone (PR) receptor activity, in good correlation with biochemical methods (Parl and Posey, 1988).

The amplification of c-erbB-2, a gene which is probably involved in tumor growth (i.e. breast cancer), may increase protein expression. This protein can be detected with MAb and polyclonal antibodies and has been associated with poor prognosis. It is found in 20-30% of breast cancers (Slamon et al., 1987; Venter et al., 1987; Barnes, 1989).

Tumor proliferative activity (TPA), as defined by the proportion of cycling cells in the total cell population, has been shown to be of prognostic value in various solid neoplasms. Ki-67 MAb immunohistochemical assays in various tumors have yielded TPA values comparable to the number of cells in S phase obtained by flow cytometric measurement (Gerdes et al., 1983, 1984; Schrape et al., 1987).

The aim of the present study is to evaluate the relationship between some different classical and recent prognostic factors in breast carcinoma.

Materials and methods

A total of 97 breast carcinoma samples from 32- to

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88-year-old women was studied between 1988 and 1992. The material was obtained from the files of the Pathology Department of the Virgen de la Cinta Hospital (Tortosa, Spain).

Some of the human breast tissue samples (surgical specimens) were frozen immediately and stored at -70 °C for Ki-67 and RH determination. The rest of the samples were formalin-fixed and paraffin-embedded; 4-5 µm-thick tissue sections were prepared for c-erbB-2 determination. One of each was stained by haematoxylin-eosin to study differentiation degree, following standard criteria (Bloom and Richardson, 1957) modified by Elston (1988), and histological type following WHO criteria (1981).

70 patients were studied for axillary metastatic lymph nodes (LN). Metastatic LN presence was classified into four different groups according to the number (0, 1-3, 4-10, more than 10).

Hormonal receptors

The immunohistochemical staining procedure for ER and PR was performed using ER-ICA and PgR-ICA monoclonal Kits (Abbott RFA) according to the manufacturer's instructions. Semiquantitative evaluations were made based on the intensity (I) of staining, evaluated from 1 to 3, and the percentage of positive cells. Histocore = (I+1) x (percentage positive cells) (range 0-400). Cases with a histocore value of over 100 were considered positive (McCarty et al., 1985).

C-erbB-2

The immunohistochemical staining procedure for c-erbB-2 oncogene expression was performed as follows. Sections were incubated overnight with 1:40 concentration of MAb NCL-CB11 (Novocastra, UK) a monoclonal antibody against the internal domain of the human c-erbB-2 protein. After that, the method was completed with an avidin-biotin-peroxidase complex (ABC) (Vector, USA) (Hsu et al., 1981) and later revealed with DAB. The intensity of staining (I= 0-2) and the number of positive cells (1= 0-24%, 2= 25-49%, 3= 50-74%, and 4= 75-100%) were evaluated as follows: I x number of positive cells (range 0-8) (Dykins et al., 1991). Positivity was essentially evaluated from the infiltrative component.

Ki-67

4-6 µm cryostat sections mounted on glass slides were air dried for an hour and placed in acetone at -20 °C for 5 to 10 minutes. After fixation the slides were incubated with 1:50 concentration of MAb Ki-67 (Dako, Denmark) for an hour, and the method was completed with the ABC mouse kit (Vector, USA). Positive cells were estimated as a percentage of the total number of counted cells (500 cells for each sample), in the areas of maximum positivity, including both the intraductal and

infiltrative components.

Statistical analysis

Spearman's test was used to analyze the data, with statistical significance for $p < 0.05$.

Results

Menstrual cycle and histological findings

As shown in Table 1, 79.4 per cent of the women were menopausal patients (6 months or more since the last menstruation). Of these, 3/4 had an invasive ductal carcinoma. If those cases corresponding to invasive ductal carcinoma with a predominant intraductal component (intraductal image in addition to the infiltrative component) were included, this percentage increased to 81.4.

Differentiation degree

About 40 per cent of the cases studied were grade I; a

Table 1. Breast cancer. Menopausal status, histological type, differentiation grade, positive axillary nodes, hormonal receptors, Ki-67 and c-erbB-2.

Menopausal status	
Premenopausal women	20 (20.6%)
Postmenopausal women	77 (79.4%)
Histological type	
Invasive ductal carcinoma with a predominant intraductal component	4 (4.12%)
Invasive ductal	75 (77.32%)
Lobular	7 (7.22%)
Medullar	5 (5.15%)
Mucinous	3 (3.10%)
Tubular	1 (1.03%)
Papillar	1 (1.03%)
Ductal + Mucinous	1 (1.03%)
Differentiation grade	
I	38 (39.2%)
II	39 (40.2%)
III	20 (20.6%)
Positive axillary nodes	
0	35 (50.0%)
1-3	16 (22.9%)
4-10	11 (15.7%)
More than 10	8 (11.4%)
Not known	27
Estrogen receptors	
Histocore > 100 (+)	63 (64.9%)
Histocore < 100 (-)	34 (35.1%)
Progesterone receptors	
Histocore > 100 (+)	40 (41.2%)
Histocore < 100 (-)	57 (58.8%)
c-erbB-2	
Positive	26 (26.8%)
Negative	71 (73.2%)
Ki-67	
Positive cells > 10%	38 (39.2%)
Positive cells < 10%	59 (60.8%)

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similar percentage were grade 2, and 20 per cent were grade 3 (Table 1). Table 2 shows the mean, standard deviation, minimum and maximum values of the parameters studied. Differentiation degrees were positively associated with c-erbB-2 and Ki-67, and negatively associated with ER and PR. There was no association between differentiation degree and the number of metastatic LN (Table 3).

Metastatic lymph node

Only one half of all the cases evaluated had metastatic LN (Table 1). Nevertheless, there was no statistical significance between this and the other variables studied (Table 3), regardless of whether individual or group comparisons were made (as shown in Table 1).

Estrogen and progesterone receptors

Specific staining was confined exclusively to the nuclei, showing different degrees of intensity and different numbers of positive cells (Figs. 1, 2). Those cases with the highest intensity had the highest number of positive cells.

65 per cent of ER and 41 per cent of PR were found in positive cases (Histocore > 100) (Table 1). ER and PR showed a negative association with c-erbB-2 and Ki-67 (Table 3).

Table 2. Mean (\bar{X}), standard deviation (SD), minimum and maximum values of differentiation grade, axillary lymph node metastases (LNM), RH, Ki-67 and c-erbB-2 in breast cancer.

PARAMETER	\bar{X}	SD	MINIMUM	MAXIMUM
Differentiation grade			1	3
LNM	2.81	4.62	0	22
ER	169.58	138.46	0	400
PR	118.22	140.41	0	400
c-erbB-2	1.38	2.49	0	8
Ki-67	11.49	13.80	1	63

Table 3. Relationship among parameters.

PARAMETERS COMPARED	SPEARMAN VALUE	P
Differentiation/LNM	0.099	0.333 (NS) ^a
Differentiation/ER	-0.570	0.000
Differentiation/PR	-0.413	0.000
Differentiation/c-erbB-2	0.254	0.012
Differentiation/Ki-67	0.514	0.000
LNM/ER	-0.060	0.555 (NS)
LNM/PR	-0.083	0.418 (NS)
LNM/c-erbB-2	-0.136	0.183 (NS)
LNM/Ki-67	0.001	0.991 (NS)
ER/PR	0.434	0.000
ER/c-erbB-2	-0.403	0.000
ER/Ki-67	-0.425	0.000
PR/c-erbB-2	-0.363	0.000
PR/Ki-67	-0.315	0.004
Ki-67/c-erbB-2	0.262	0.009

^aNS: not significant.

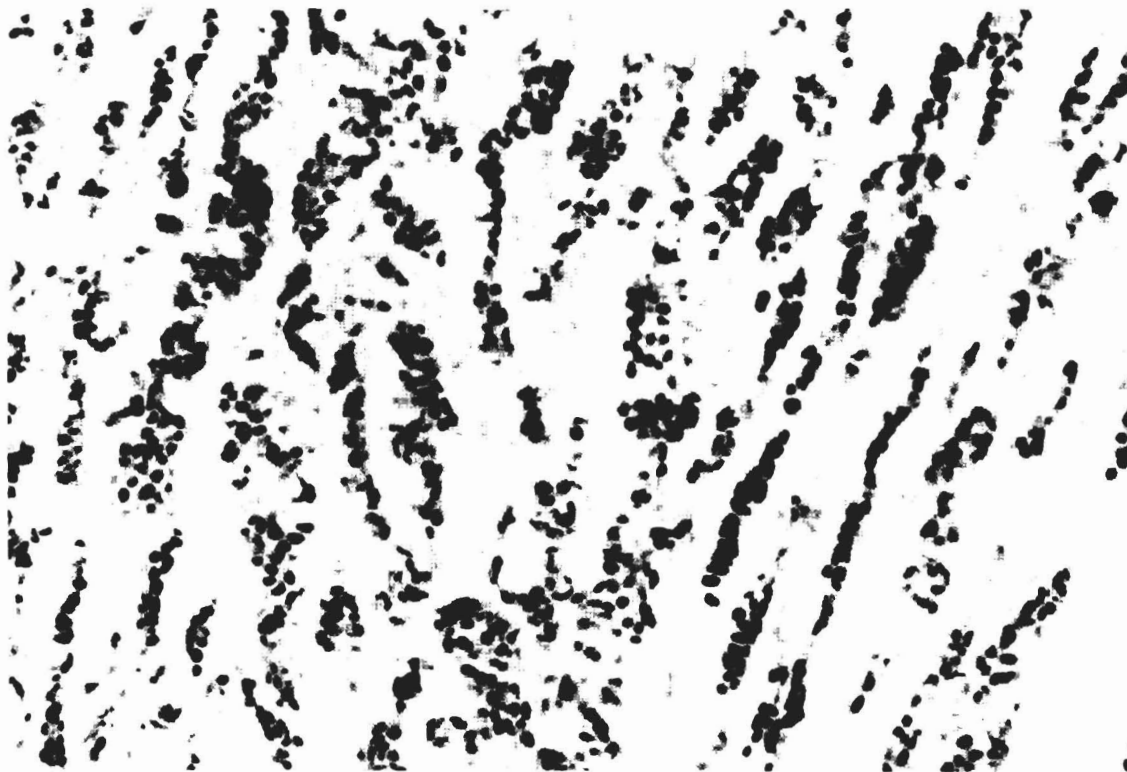


Fig. 1. Invasive lobular carcinoma. Estrogen receptors. Intensity 3 in almost all the cells. PAP. x 250

C-erbB-2

Samples were classified as immunoreactive when distinct cell membrane staining was observed (Fig. 3). Different degrees of positivity were observed in 27 per cent of the cases. The seven cases of lobular carcinoma were negative. Of the 4 cases of invasive ductal carcinoma with a predominant intraductal component, two were positive, basically in the intraductal component, corresponding to comedocarcinoma; the other two, with an intraductal cribriform pattern, were negative.

C-erbB-2 is positively associated with differentiation degree and negatively associated with hormonal receptors.

Ki-67

Specific staining was confined exclusively to the nuclei (Figs. 4, 5). Staining results were recorded as low cell proliferation index when the number of positive cells was less than 10 per cent (40 per cent of the cases). The cell proliferation index < 1 was found in 22.6 per cent of the cases. According to the histological type the range for this rate in lobular carcinoma was between 1 and 4 per cent, while in medullar carcinoma this index was higher than 15 per cent. Ductal carcinoma presented variable values.

Ki-67 is negatively associated with ER and PR and positively associated with c-erbB-2.

Discussion

Few studies have been made to establish the relationship between classical prognostic factors (differentiation degree, metastatic lymph node presence, and HR) and some new prognostic factors (TPA and c-erbB-2 gene amplification). In one of them, 1073 cases were studied using different determination techniques (for c-erbB-2 using Southern and Western Blot, HR through biochemical procedures and cell proliferation using S phase). The same conclusions were reached (Ciocca et al., 1992). Bacus et al. (1989) studied a series of 50 cases using a similar methodology: ER and PR showed a weak correlation with histological grades, and quantification of Ki-67-positive nuclear area provided an objective method for assessing tumor cell growth fraction.

Differentiation degree is one of the most important histological parameters in breast cancer prognosis. In our study, Ki-67 and c-erbB-2 showed a significant correlation (positive association), just as hormonal receptors (negative association) did with differentiation degree. All of these factors together (i.e., a high differentiation degree, ER and PR absence, high positivity of Ki-67 and c-erbB-2 expression) indicate patients with a poor prognosis. Even though the presence of metastatic LN is a poor prognostic factor, there seems to be no relationship between it and ER and PR, Ki-67 or c-erbB-2. The reason for this could be that the presence of metastatic LN is just a time indicator of tumor evolution,

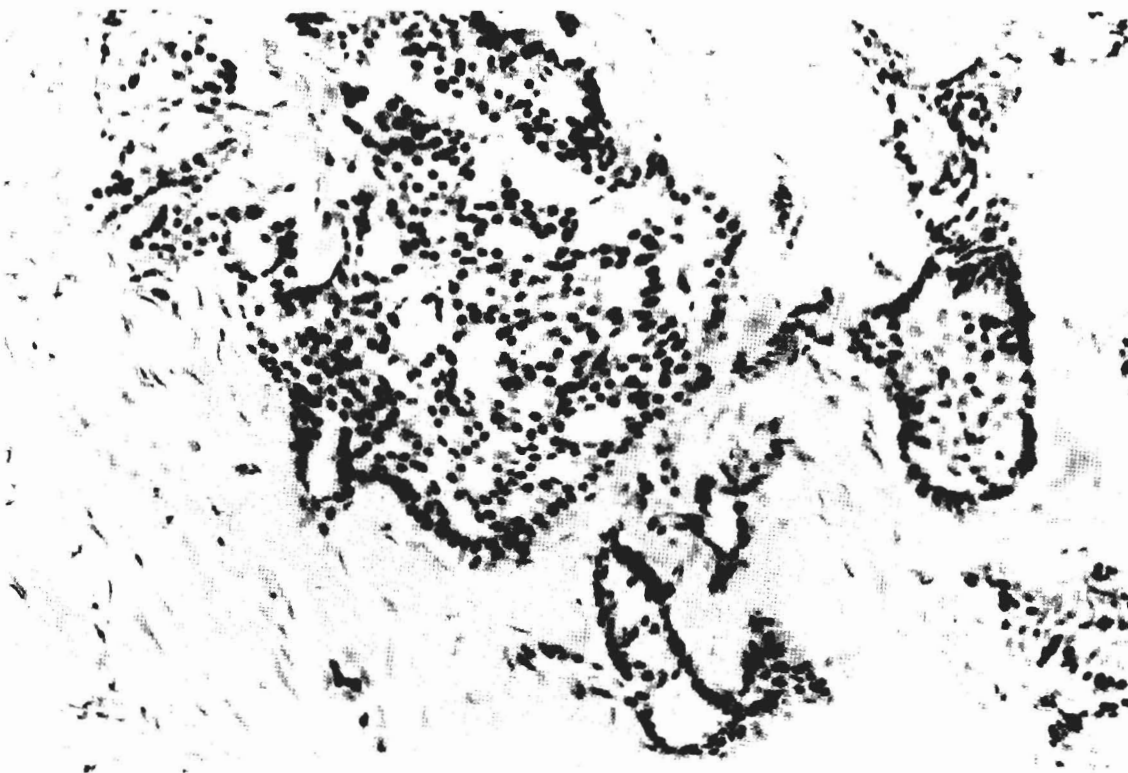


Fig. 2. Invasive ductal carcinoma. Progesterone receptors. Intensity 3 in 75% of the cells. PAP. x 250

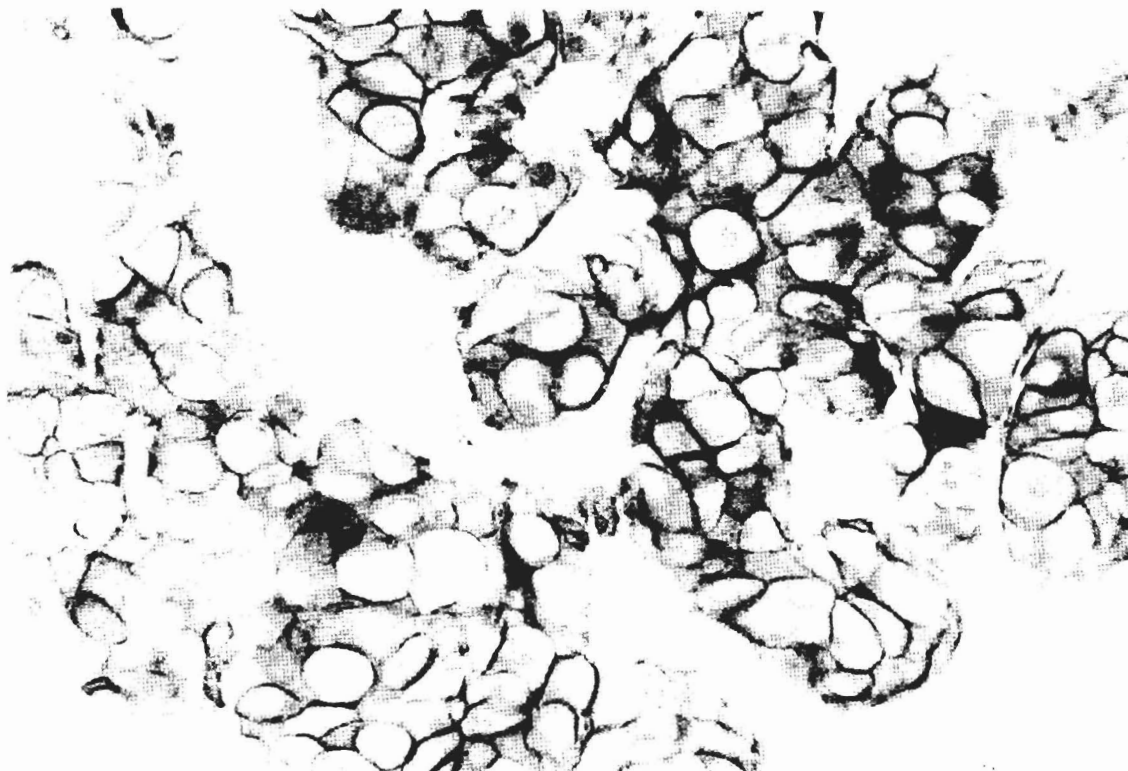


Fig. 3. Invasive ductal carcinoma. c-erbB-2. High positivity (2) membrane staining. ABC. x 400

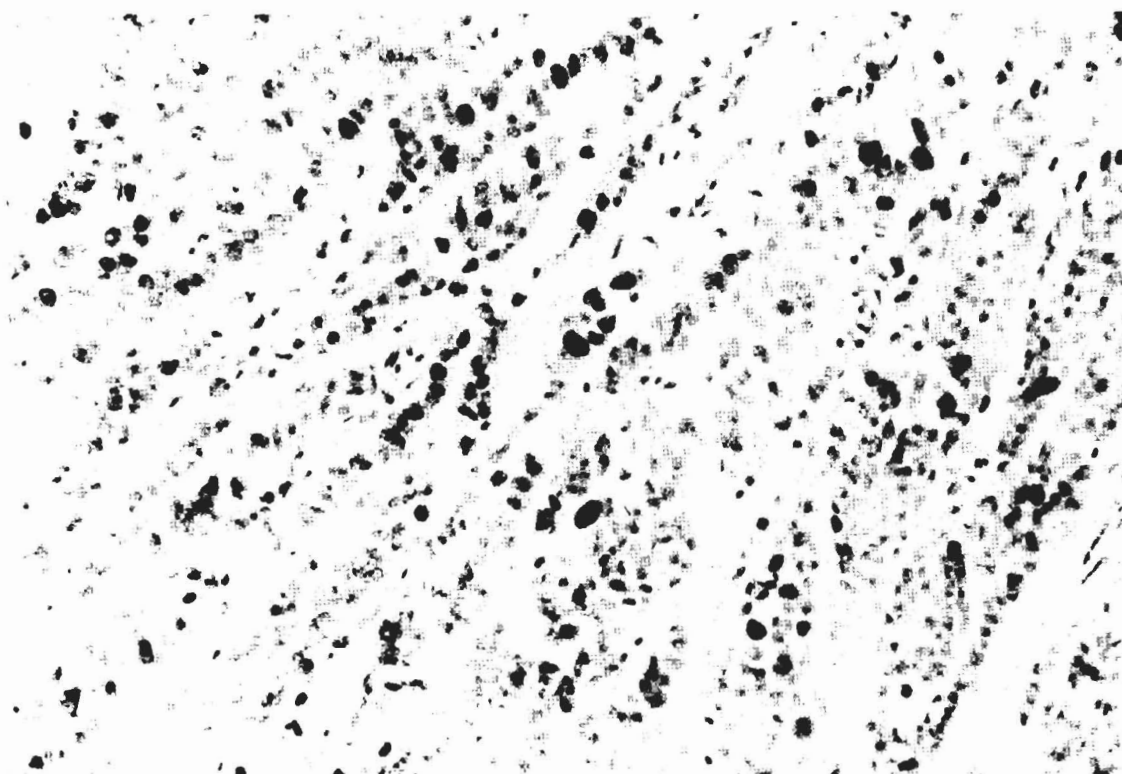


Fig. 4. Invasive ductal carcinoma. Ki-67. High proliferation factor (48%) ABC. x 250

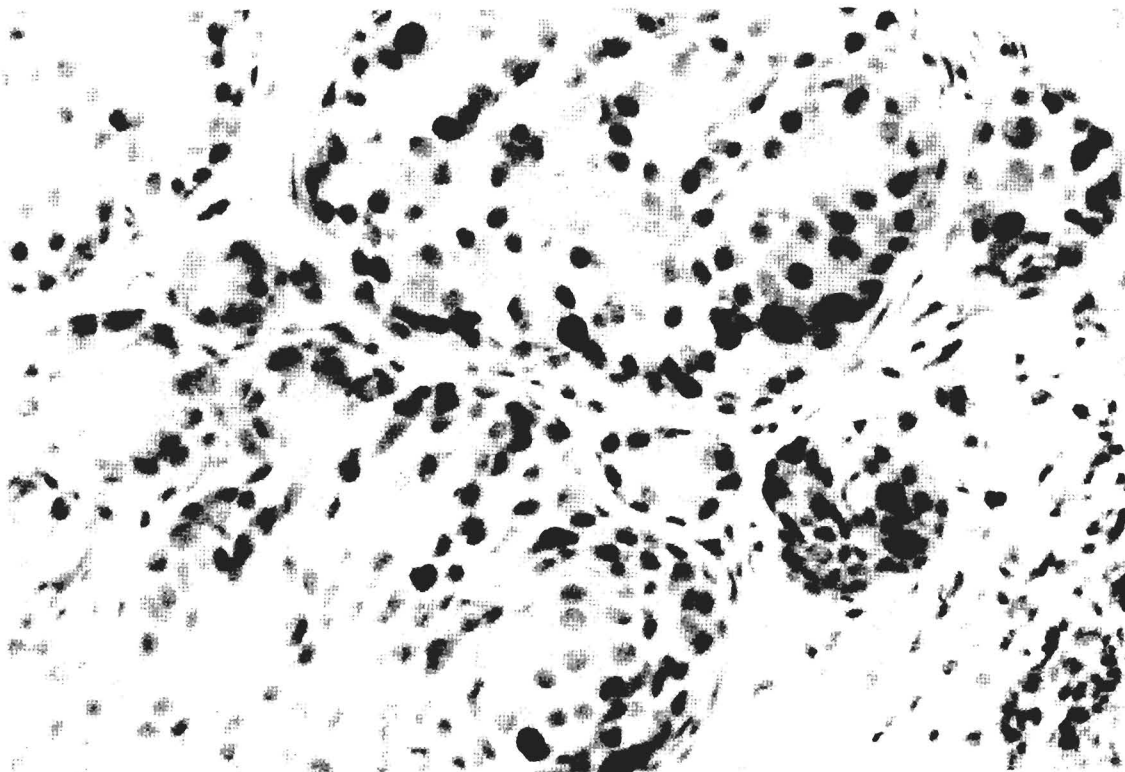


Fig. 5. Invasive ductal carcinoma. In situ component. Low cell proliferation index (4%). ABC. x 250

rather than a biological state (Mittra and MacRal, 1991).

Thirty per cent of the patients with negative metastatic axillary LN eventually relapse, and occasionally die of the illness (Van de Velde et al., 1986; Fisher et al., 1990). In recent years efforts have been made in breast cancer treatment to choose that subpopulation whose lesions are likely to recur or metastasize, requiring appropriate hormonal therapy. ER and PR determinations have been widely used to this end (Sigurdsson et al., 1990). Immunohistochemical assays with MAb methods have been accepted as an alternative to ER and PR determinations (McCarty et al., 1985; Von Kleist et al., 1988).

Twenty five to 30 per cent of the cases are positive to c-erbB-2 depending on the case and histological type (Schimmelpenning et al., 1992). The results according to histological type are different. While ductal carcinoma in situ shows positivity in 44 per cent of cases, infiltrating ductal carcinoma is only positive in 16-20 per cent, and lobular carcinoma is almost always negative (Gusterson et al., 1988).

C-erbB-2 over-expression has been noted to be a poor prognostic factor, especially when there is metastatic LN presence (Dykins et al., 1991), but not always (Rilke et al., 1991). There is also a relationship between c-erbB-2 and differentiation degree (Corbett and Dykins, 1991; Rilke et al., 1991). Over-expression of c-erbB-2 in a group of breast cancers with other markers of good prognosis (tumor size of less than 3 cms, positive HR and negative LN) presages early relapse (McGuire et al.,

1990) and responds less well to conventional chemotherapy (CMF) (Gusterson, 1992).

Ki-67 is not only a good tumor proliferation indicator, but a good parameter of possibility of relapse (Veronese et al., 1993). Cases with a positivity of over 10 per cent have a high rate of recurrence, and when this percentage rises to more than 15, patients usually die from this illness. Patients with a percentage of less than 4 do not usually relapse (Sahin et al., 1991). These comments do not apply to medullary carcinoma. Ki-67 shows a positive association with differentiation degree and negative association with ER and PR (Raymond and Leong, 1989; Leonardi et al., 1992).

Although an appropriate semiquantitative evaluation is a method which is reproducible, easy to apply and extremely informative for any pathology laboratory, there is no doubt that the introduction of Image Analysis can provide a more objective quantification of these markers (Esteban et al., 1993).

In conclusion, there is a group of patients with a high differentiation degree, without ER and PR, an over-expression of c-erbB-2 and a high cell proliferation index whose lesions are likely to recur or metastasize, but further clinical studies to determine the survival rate should be made.

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