Ductal Carcinoma In Situ of the Breast

Histologic Categorization and Its Relationship to Ploidy and Immunohistochemical Expression of Hormone Receptors, p53, and c-*erb*B-2 Protein

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Background. **Ductal carcinoma in situ (DCIS) of the breast has been diagnosed increasingly since the advent of mammography. However, the natural history of these lesions remains uncertain. Ductal carcinoma in situ of the breast does not represent a single entity but a heterogeneous group with histologic and clinical differences. The histologic subtype of DCIS seems to have an influence on its biologic behavior, but there are few studies correlating subtype with biologic markers.**

Methods. **The authors studied a consecutive series of 40 cases of DCIS and after its histologic categorization verified its relationship with ploidy using image analysis and analyzing estrogen receptor (ER), progesterone receptor (PR), p53 and c-*erb*B-2 expression using immunohistochemistry.**

Results. **The three groups proposed according to the grade of malignancy were correlated significantly with some of the additional parameters studied, including aneuploidy and c-*erb*B-2 expression. Aneuploidy was detected in 77.5% of cases of DCIS mainly in high and intermediate grade subtypes (100% and 80% vs. 35.7% in low grade) whereas immunoreactivity for c-*erb*B-2 was detected in 45% of cases of DCIS mainly in the high grade group. Expression of ER and PR were observed frequently in this study (63.9% and 65.7% respectively), but without correlation with the histologic subtype of DCIS, although we found a somewhat significant association between high grade DCIS and lack of ER. p53 protein expression was detected in 36.8% of these cases, but no relationship between this expression and histologic subtype or grading of DCIS was found.**

Conclusions. **These results provide further evidence for the morphologic and biologic heterogeneity of DCIS. Besides histologic classification and nuclear grading, some biologic markers such as aneuploidy and c-*erb*B-2 expression constitute additional criteria of high grade of malignancy. *Cancer* 1995;75:2123–31.**

Key words: breast ductal carcinoma in situ (DCIS), DCIS subtypes, ploidy, hormone receptors, p53, c-*erb*B-2 expression.

The widespread use of mammographic screening has increased the diagnosis of preinvasive breast carcinomas.[1,2] However, the natural history of these lesions remains unknown.[1,3,4] This has led to difficulties in determining a logical care policy for the increasing number of patients detected with ductal carcinoma in situ (DCIS). Thus, it is important not only to diagnose these lesions accurately, but also to identify prognostic features for relapse or potential invasiveness.

Although the evaluation of tumor size and margin involvement appears valuable for the current care of patients, the histologic subtypes of DCIS seem to have influence on its biologic behavior.

Until the last few years, most emphasis in the histologic classification for DCIS had been based on architectural features, and these tumors were divided into five major patterns: solid, comedo, cribriform, micropapillary, and papillary.[4–8] Recently, it was demonstrated that the cytonuclear features of DCIS should be incorporated in its pathologic evaluation, although their full significance has not been established.[3,6]

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In invasive breast carcinomas, several biologic markers of prognostication have been joined to the classic parameters obtained by morphologic evaluation. Some of these markers have been studied in isolated form in series of DCIS.[8–14]

The current study examines the frequency of hormone receptor positivity, c-*erb*B-2 and p53 expression, and nuclear DNA content in a series of patients with pure DCIS, and the associations between these features and the histologic classification of DCIS.

Patients and Methods

Patient Selection

Routinely formalin fixed, paraffin embedded breast tissues from 40 patients with pure DCIS, drawn from the files of the Department of Pathology of Instituto Português de Oncologia Francisco Gentil-Porto, between the years of 1985 and 1993 were investigated.

Pathologic Analysis

For each patient, all of the slides were reviewed, and a representative paraffin embedded block was chosen for immunohistochemistry and DNA analysis. According to the architectural and nuclear established criteria that allow the subdivision of the subtypes of DCIS in different grades of malignancy,[3,6–8] we divided them into three groups—low, intermediate (noncomedo with necrosis), and high grade—evaluating for each one the histologic subtype, nuclear grade, and presence or absence of necrosis.

The histologic subtype was classified according to the predominant architectural pattern as comedo, solid, cribriform, micropapillary, or papillary type, when such a pattern corresponded to more than 75% of the tumor. When no pattern predominated, the tumor was classified as mixed.

Nuclear grade was defined as Grades 1–3 in order of increasing pleomorphism with regard to the invasive carcinoma.[15] Briefly, grade designation was made according to pleomorphism, size, and presence of nucleoli. Nuclear size was assessed in a semiquantitative manner by comparative evaluation with nuclei of adjacent normal breast duct as small (1–2 × size), intermediate (3–4 × size), or large (5 × size or more). Grade 1 nuclei was characterized by monomorphism, small or intermediate size, and inconspicuous nucleoli. Grade 2 nuclei showed moderate pleomorphism, small or intermediate size, and often was characterized by evident nucleoli. Grade 3 nuclei showed marked pleomorphism, intermediate or large size, and frequently contained multiple and conspicuous nucleoli.

Necrosis was considered to be present when small or large areas of comedo type necrosis were seen. Isolated necrotic cells were not considered.

Immunohistochemistry

For immunostaining, the avidin-biotin-peroxidase complex (ABC) method was used. Briefly, the sections from formalin fixed material were cut, dewaxed, and treated with 0.3% hydrogen peroxide (H_2O_2) in methanol for 30 minutes to quench the endogenous peroxidase activity. The slides were briefly rinsed in distilled water and incubated for 10 minutes at 750 W (domestic microwave) in 10 mM citrate buffer in a thermoresistant container. Distilled water and buffer were added periodically to the container to prevent drying during the incubation process. The slides were cooled in buffer for 20 minutes (to room temperature), washed in distilled water, and rinsed in phosphate buffered saline (PBS). The primary antibodies were applied to the sections and incubated overnight at 4°C. This was followed by incubation with a 1:100 dilution of biotin labeled anti-mouse secondary antibody for 30 minutes and ABC for 60 minutes. Careful rinses were done with PBS between each step of the procedure. The color was developed with diaminobenzidine, and the sections were lightly counterstained with hematoxylin, dehydrated, and mounted.

The primary antibodies used were: estrogen receptor, clone ER1D5, undiluted (Immunotech SA, Marseille, France); progesterone receptor, clone PR10A9, diluted 1:20 (Immunotech SA); and p53 protein, clone DO-7, diluted 1:50 (Dakopatts, Copenhagen, Denmark). For c-*erb*B-2 immunostaining we used the antibody from Dako (Dakopatts, Denmark) diluted 1:150, and incubation was done at room temperature that did not require microwave processing. Negative control for the immunostaining was performed by substituting the primary antibody with a mouse myeloma protein of the same subclass and concentration of the primary antibody. As a positive control, we used sections from cases of invasive breast carcinoma known to express estrogen receptor (ER), progesterone receptor (PR), p53, and c-*erb*B-2. Tumor cells showing distinct brown nuclear staining were interpreted as positive for ER, PR, and p53. Initially, ER and PR were assessed using an H score system[13,14]; the final score for each was the sum of the percentage of neoplastic cells staining weakly plus twice the percentage of tumor cell nuclei staining moderately plus three times the percentage of tumor cell nuclei staining strongly for ER and PR. The possible scores ranged between 0 and 300. However, for the statistical analysis, all cases that showed at least focal ER and PR positivity were considered positive.[14] Cases with 5% or

more tumor cell nuclei showing p53 immunostaining were scored as positive.[14] Tumor membrane immunoreactivity, either homogeneous or heterogeneous, was used as the sole criterion of c-*erb*B-2 overexpression.

DNA Measurements

Paired sections were cut from each selected paraffin block, dewaxed, and rehydrated. One slide from each block was stained by the Feulgen technique (acid hydrolysis 5 N hydrochloric acid at room temperature for 60 minutes). The other section was stained with hematoxylin and eosin. A pathologist (F.C.S.) identified and marked microscopic fields for image analysis on the hematoxylin and eosin stained slides.

The DNA measurements were performed using a television based image analysis system. It is based on a light microscope (plan objective × 40) equipped with a video-CCD camera connected to a microcomputer.

The cytophotometric measurements of stained cell nuclei were performed at a wavelength of 546 ± 10 nm. Areas corresponding to those outlined on the hematoxylin and eosin stained sections were identified in the Feulgen stained sections. The lymphocyte nuclei from each section served as the internal control. From each patient, 200 nuclei of the proliferative epithelial lesions and 50 control nuclei were measured. DNA histograms were generated by plotting nuclear optical density of Feulgen stained DNA versus the number of nuclei. For each patient, the G0/G1 peak visually identified, the mean, standard deviation (SD), and coefficient of variation values were calculated. The control coefficient of variation provided an indication of overall precision of the imaging technique. Aneuploid peaks were those exceeding the internal control lymphocyte (diploid) G0/G1 peak mean by 2 SD. The percentage of nuclei exceeding 5 n DNA content, as defined by the control cell 2 n DNA content, also was counted.

Using a modification of Auer's classification scheme, the histograms were classified into "euploid" (types I and II) or "aneuploid" (types III and IV).[16,17] A type I histogram is characterized by a single distinct peak in the diploid or near-diploid region of normal cells with a "tumor" G0/G1 DNA mean value within 2 SD of the control cell G0/G1 DNA mean values. Type II populations show a distinct modal value in the tetraploid or near-tetraploid region or have two well defined peaks around the 2-n and 4-n regions, presumably representing overlapping nuclei or cells arrested in the G2 phase. Type III histograms have a "tumor" G0/G1 peak exceeding the control cell G0/G1 peak mean by 2 SD of the control cell peak. Type IV histograms show a pronounced and irregular aneuploidy, with DNA amounts ranging from 2 n to values exceeding 6–8 n.[17]

Statistical Analysis

Statistical analysis were performed using the chi-square test and, for small numbers, Fisher exact test. A level of $P < 0.05$ was considered significant.

Results

Patient Details

The patients' average age at the time of diagnosis was 55 years (range, 37–87 years). At presentation 11 patients had symptomatic disease (palpable lump or nipple discharge), and 25 patients had asymptomatic disease (lesions detectable only by mammography). For four patients, presentation was unknown. The size of the lesions varied between 4 and 50 mm (median, 16.3 mm).

Histologic Findings

The data on histologic patterns, estrogen and progesterone receptors, p53 and c-*erb*B-2 immunoreactivity, and DNA nuclear content of DCIS are summarized in Tables 1, 2, and 3 and in Figures 1, 2, and 3.

DCIS Subtypes

Analysis of DCIS with regard to frequency of the predominant growth pattern showed that the comedo and mixed types were the most common subtypes, with each occurring in 32.5% of all patients, followed by cribriform, in 7 (17.5%) patients; micropapillary, in 4 (10.0%) patients; solid, in 2 (5.0%) patients; and papillary, in 1 (2.5%) patient. In the mixed lesions, cribriform was the prevalent pattern, being combined in most of the patients with the micropapillary subtype.

Nuclear Grade

Nuclear Grade 3 was seen in 18 (45%) patients with DCIS, including all with the comedo and micropapillary patterns and 1 with mixed pattern. Nuclear Grade 2 was seen in 8 (20%) patients, all of whom had mixed pattern. The remain 14 (35%) patients with DCIS were classified as having nuclear Grade 1, including all patients with the pure cribriform, solid, and papillary architectural patterns and 4 with the mixed subtype.

Necrosis

Necrosis was present in 24 (60%) of our patients with DCIS, including all with the comedo subtype, 1 with

Table 1. Histologic Grade of Ductal Carcinoma in Situ

	High grade	Intermediate grade	Low grade
Nuclear grade	3	1/2	1/2
Necrosis	Usually present Often prominent	Present	Absent
Growth pattern (found in our series)	Comedo (n = 13) Micropapillary (n = 4) Mixed (n = 1)	Mixed (n = 7) Cribriform (n = 3)	Cribriform (n = 5) Mixed (n = 4) Solid (n = 2) Papillary (n = 1)

the micropapillary, 3 with the cribriform, and 7 with the mixed subtypes.

Histologic Grade

In Table 1 we summarize the criteria for classification of DCIS according to histologic grade. Based on the nuclear grade and in the presence or absence of necrosis, the patients were classified having the following grades of DCIS:

1. High grade DCIS: 18 patients (45% of the tumors). High nuclear grade was present in all patients. The histologic subtypes were: comedo (13 patients), micropapillary (4 patients), and mixed (1 patient). Necrosis was present in 14 patients (13 comedo and 1 micropapillary) and absent in 4 patients (3 micropapillary and 1 mixed).
2. Intermediate grade DCIS: 10 patients (25% of the tumors). In this group, we included all the noncomedo DCIS with necrosis. The histologic subtypes seen in this category were: cribriform (three patients) and mixed (seven patients). Nuclear grade was 2 in six patients and 1 in four patients.
3. Low grade DCIS: 12 patients (30% of the tumors). In this group were 10 patients with nuclear Grade 1 and 2 patients with nuclear Grade 2, all of whom had no necrosis. The histologic subtypes seen were:

Table 2. Histologic Subtype/DNA, c-*erb*B-2, ER, PR, and p53

Histologic subtype	DNA		c-*erb*B-2		ER		PR		p53	
	Eup	*Anp*	*Pos*	*Neg*	*Pos*	*Neg*	*Pos*	*Neg*	*Pos*	*Neg*
Comedo	0	13	11	2	5	6	6	5	5	7
Cribriform	4	3	0	7	5	1	5	1	2	5
Micropapillary	0	4	4	0	1	3	2	2	2	2
Solid	2	0	0	2	1	1	2	0	0	2
Papillary	0	1	0	1	1	0	1	0	0	1
Mixed	3	10	3	10	10	2	7	4	5	7
Total	9	31	18	22	23	13	23	12	14	24
P value	0.005		0.00002		0.16		0.62		0.77	

ER: estrogen receptor; PR: progesterone receptor; Eup: euploid; Anp: aneuploid; Pos: positive; Neg: negative.

Table 3. Histologic Grade/DNA, c-*erb*B-2, ER, PR and p53

Histologic grade	DNA		c-*erb*B-2		ER		PR		p53	
	Eup	*Anp*	*Pos*	*Neg*	*Pos*	*Neg*	*Pos*	*Neg*	*Pos*	*Neg*
High	0	18	15	3	7	9	9	7	7	10
Intermediate	2	8	1	9	7	1	7	1	5	4
Low	7	5	2	10	9	3	7	4	2	10
Total	9	31	18	22	23	13	23	12	14	24
P value	0.0008		0.00005		0.067		0.30		0.16	

ER: estrogen receptor; PR: progesterone receptor; Eup: euploid; Anp: aneuploid; Pos: positive; Neg: negative.

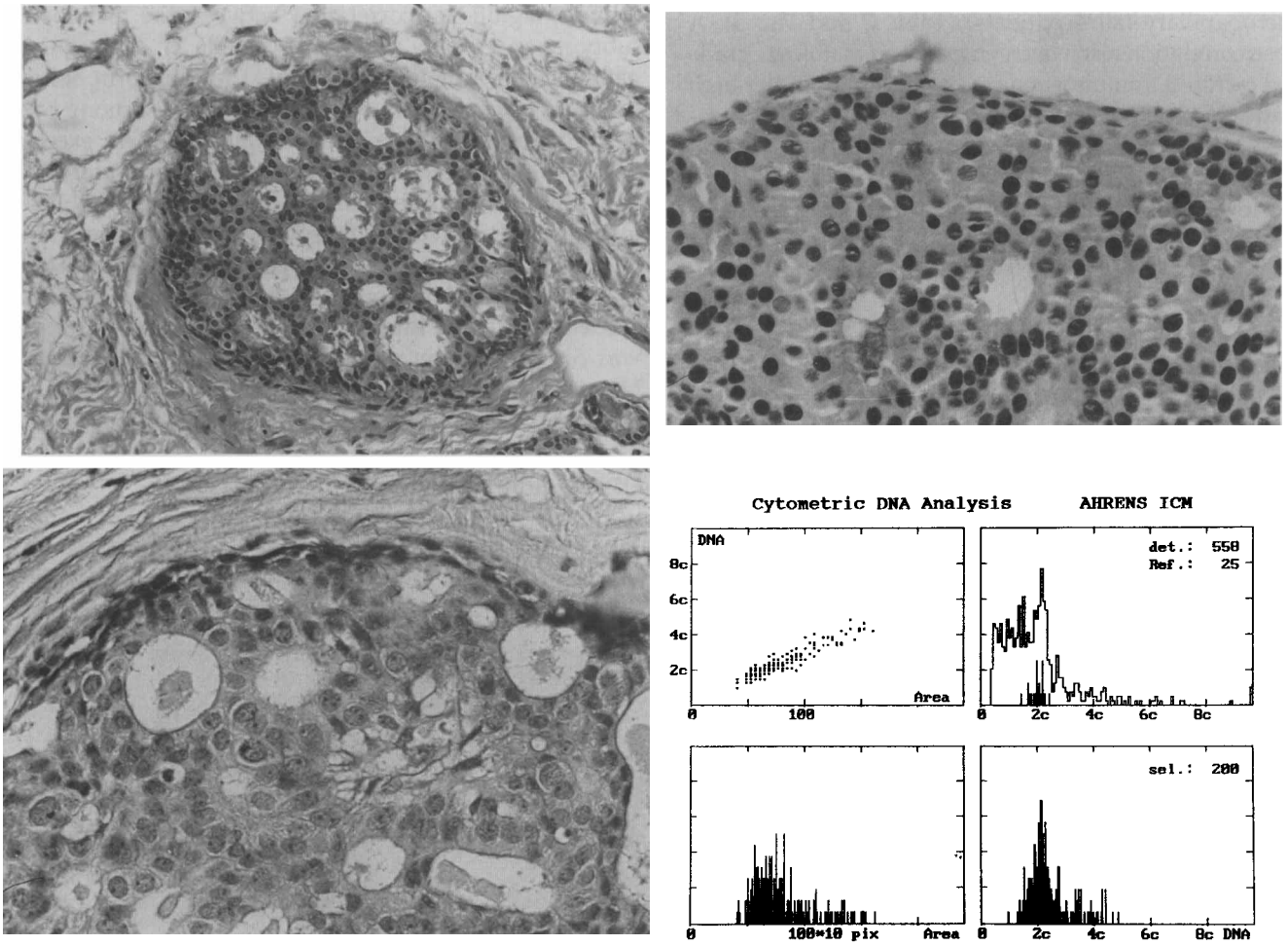

Figure 1. Summarized findings in low grade DCIS: (top left) cribriform architectural pattern (H & E, original magnification ×400); (top right) strong nuclear immunoreactivity for ER (ABC, original magnification ×560); (bottom left) negativity for c-*erb*B-2 protein (ABC, original magnification ×560), and (bottom right) diploid DNA profile, Auer's type I histogram.

cribriform (four patients), papillary (one patient), solid (two patients), and mixed (five patients).

Estrogen and Progesterone Receptors

ER and PR were available in 36 and 35 patients, respectively. We found a high frequency of ER (63.9%) and PR (65.7%) positivity in our patients with DCIS (Table 2). This positivity was not correlated with the histologic subtype, but a near significant difference between the three histologic grades considered was found ($P = 0.06$), suggesting that high grade DCIS lack ER. This evidence is reinforced by the significant lack of ER positivity in the high grade DCIS ($P = 0.02$) when compared with the positivity found in the intermediate and low grade DCIS. In fact, only 7 of 16 (43.7%) patients with high grade DCIS have ER positivity, whereas such positivity was present in 16 of 20 (80%) patients with intermediate and low grade DCIS (Figs. 1 and 2 and Table 3).

p53 Immunoreactivity

Data regarding p53 were available for 38 patients. Fourteen (36.8%) of these patients were scored as positive (Table 2). Although we observed a tendency for increased frequency of p53 protein expression in high grade tumors (7 patients, 41.2%), the difference was not statistically significant (Table 3). We also did not observe correlation between p53 expression and the histologic subtype of DCIS.

c-erbB-2 Immunoreactivity

Data regarding c-*erb*B-2 were available for all 40 patients. Eighteen of 40 (45.0%) patients with DCIS had membrane staining to c-*erb*B-2. There was a strong correlation between c-*erb*B-2 expression and the histologic subtypes of DCIS, with predominance of positivity in the comedocarcinomas (11 of 13 patients, 84.6%) and

micropapillary (all 4 patients) (Table 2 and Fig. 3). A close correlation also was seen between histologic grade and c-*erb*B-2 immunostaining ($P < 0.0005$). In the high grade DCIS, 15 of 18 (83.3%) patients had tumor samples stain positively, whereas only 1 of 10 (10%) patients in the intermediate group and 2 of 12 (16.7%) in the low grade group had positivity for c-*erb*B-2 (Table 3).

DNA Nuclear Content

Aneuploid DNA profiles were found in 77.5% of the patients with DCIS (Auer's histograms type III and IV). Among the lesions, 12.5% were tetraploid (type II histogram) and 10% were diploid (type I histogram). There was not a statistically significant difference among the histologic subtypes of DCIS and histogram types; however, when we divided the patients into groups of those with euploid and those with aneuploid profiles, we found a significant correlation between the histologic subtypes and ploidy ($P = 0.005$) (Table 2). Among the patients with euploid profiles, only the cribriform, solid, or mixed type of DCIS was found, whereas among patients with aneuploidy profiles, all subtypes, except solid DCIS, were found. Comedocarcinomas were all aneuploid and showed a high frequency of type IV histograms (Fig. 3). We also found a significant correlation between histologic grade and ploidy ($P = 0.0008$) (Table 3). Aneuploidy was found in all patients with high grade DCIS; in the intermediate grade, aneuploidy also was common, being present in 8 of 10 (80%) patients. However, of the low grade tumors, 7 of 12 (58.3%) patients had euploid profiles.

Discussion

It recently has been emphasized that carcinomas in situ of the breast do not represent a single entity. In addition

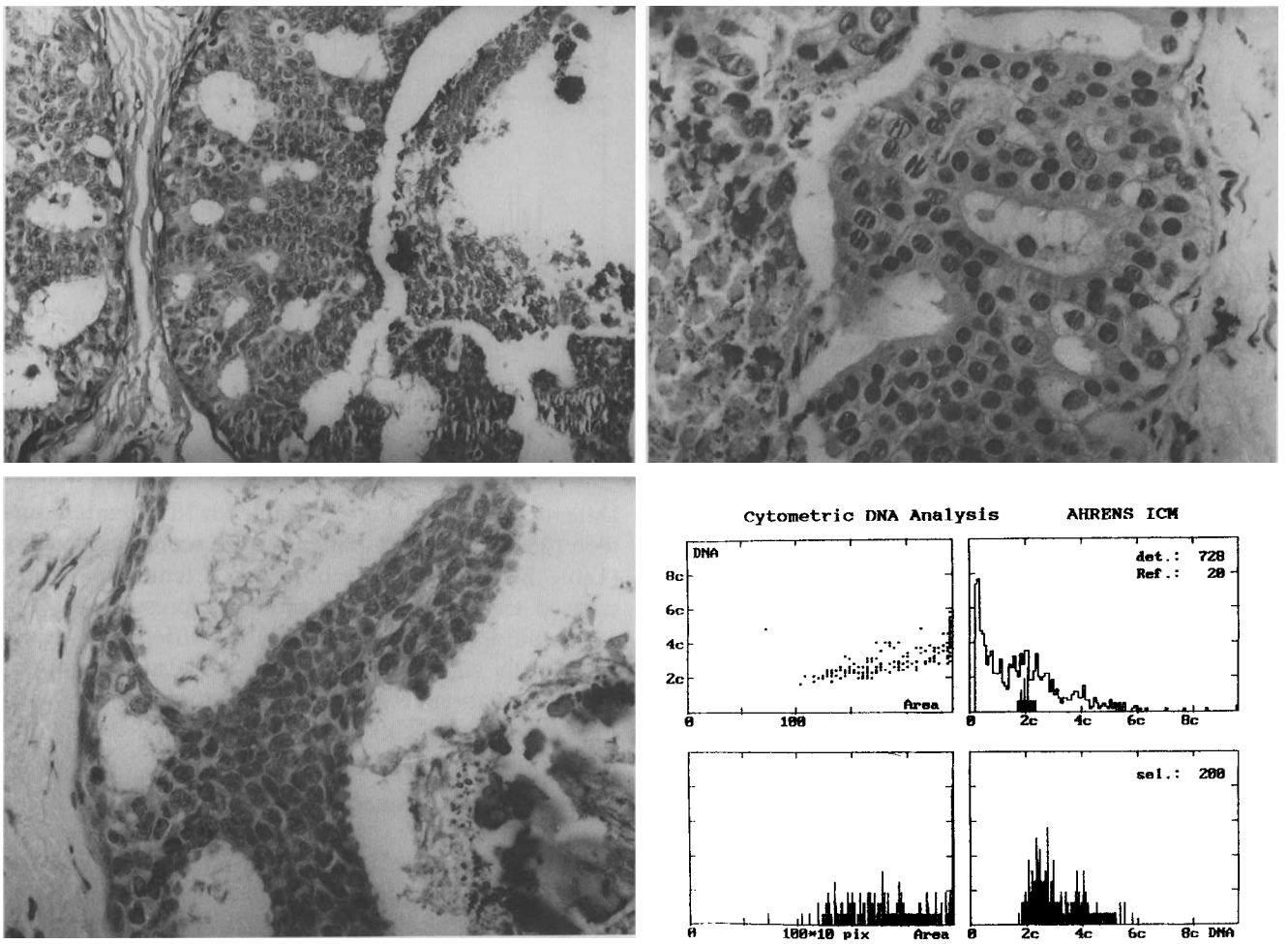

Figure 2. Summarized findings in intermediate grade DCIS: (top left) cribriform architectural pattern with necrosis (H & E, original magnification ×500); (top right) nuclear immunoreactivity for ER (ABC, original magnification ×560); (bottom left) negativity for c-*erb*B-2 protein (ABC, original magnification ×500), and (bottom right) aneuploid DNA profile, Auer's type III histogram.

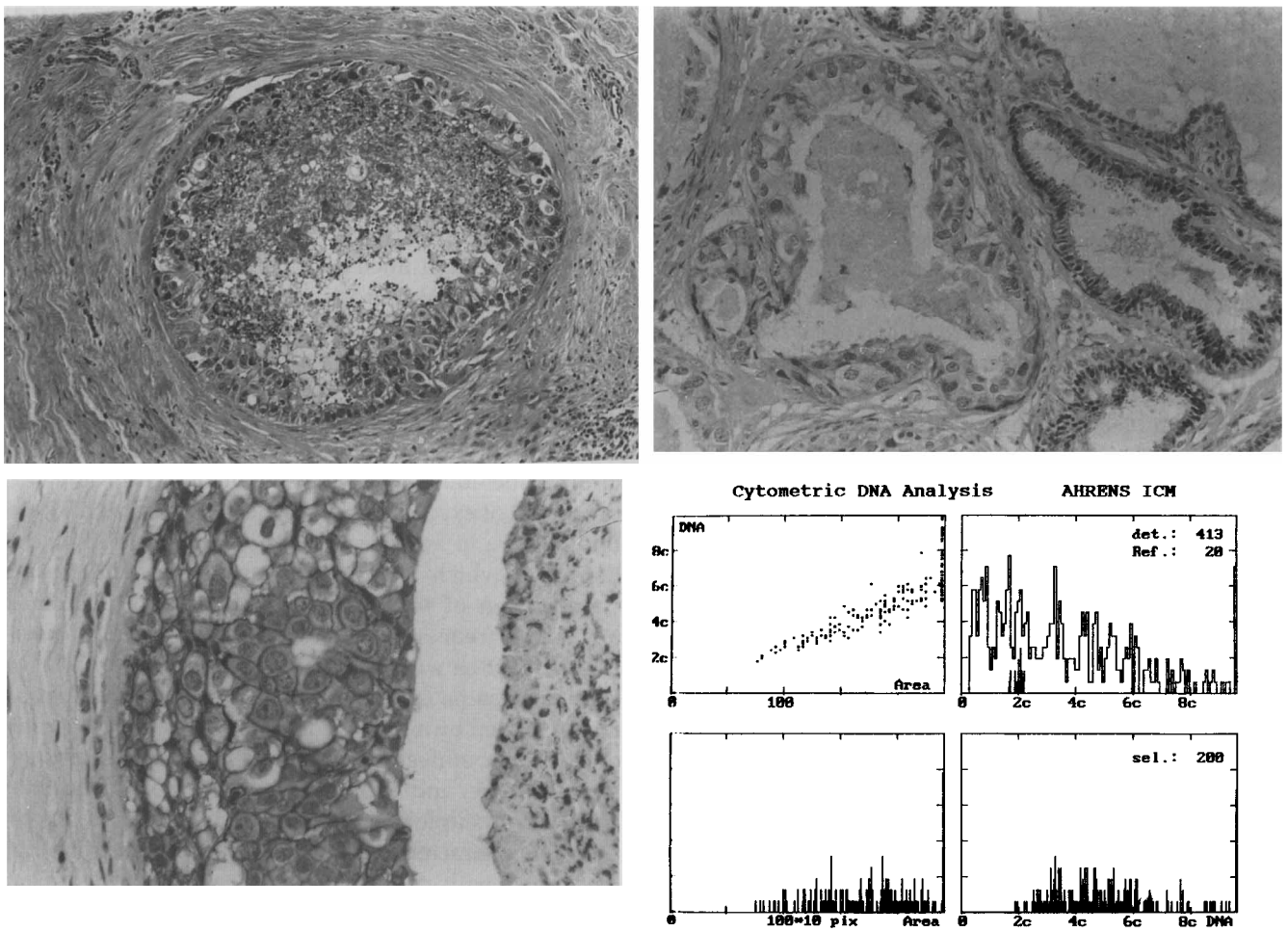

Figure 3. Summarized findings in high grade DCIS: (top left) comedo architectural pattern (H & E, original magnification ×400); (top right) negativity for ER, note some benign nuclei displaying immunoreactivity (ABC, original magnification ×500); (bottom left) membrane positivity for c-*erb*B-2 protein (ABC, original magnification ×560), and (bottom right) aneuploid DNA profile, Auer's type IV histogram.

to the classic differences between ductal and lobular carcinomas in situ, there are histologic and clinical differences among the DCIS that support the concept that these lesions represent a heterogeneous group.[3–8] Most studies showing this heterogeneity deal with architectural patterns[8] and nuclear grade of the DCIS.[3,6]

Our study confirms the histologic heterogeneity of DCIS. The following types were the most common: comedo, cribriform, and mixed. Clinical differences also were seen with regard to this morphologic heterogeneity because most of the DCIS comedo type tumors were not palpable and were discovered only when microcalcification was noted on mammography (data not shown). Two groups of DCIS showed interesting results and deserve special comments: mixed and micropapillary carcinomas. Our findings showing that 32.5% of the DCIS are mixed type are in accordance with the findings reported by Lennington et al.,[8] who found 33% of mixed type DCIS in their series. These findings support the evidence regarding the frequency of lesional heterogeneity of DCIS. In the series by Lennington et al.,[8] the mixed DCIS are more frequently low grade lesions, whereas in our series, 8 of 13 (61.5%) lesions were of high (one patient) or intermediate (seven patients) histologic grade and were aneuploid. However, only three (23%) of the mixed DCIS showed immunoreactivity for c-*erb*B-2, which is in contrast with the immunoreactivity of the comedo type, for which 84% of patients had c-*erb*B-2 expression. In our series, all patients with the other subtype (micropapillary DCIS) had tumors of high nuclear grade that were aneuploid and expressed c-*erb*B-2; three of four tumors did not express ER, and necrosis was found in one patient. These findings contrast with those reported in the literature, where most micropapillary DCIS are described as low grade.[6,7] The need to identify and separate the micropapillary pattern was demonstrated in our series by the large size and multicentricity of the tumor in three of

four patients (data not shown), which confirms the findings of Bellamy et al.[3]

Efforts to classify DCIS into a small number of groups with regard to the grade of malignancy are widespread, with researchers using several different criteria with the same objective: simplification and reproducibility. The following are common examples proposed in the literature: large cells and small cells, comedo and noncomedo, high grade and low grade; well, intermediate, and poorly differentiated.[3,4,6–8,18] Despite the unanimous recognition that all classic comedo type DCIS are constituted by large cells, the use of cell size as criteria for any classification must be avoided, because inherent subjectivity makes the reproducibility much more difficult, if not impossible. In fact, some DCIS that express other criteria of high grade malignancy, such as high nuclear grade, may be composed of cells that are not as large as the ones of comedo DCIS but not as small as the ones of cribriform DCIS, a finding we saw in some of our patients with micropapillary and mixed DCIS.

The classification in comedo and noncomedo types is operative and can be used with a high degree of reproducibility. However, it must be recognized that there are some types of noncomedo DCIS that must be seen as high grade, either because of the cytologic criteria or because they express some other markers with the same significance, such as immunoreactivity for c-*erb*B-2 and aneuploidy. All of our patients with pure micropapillary type and one of our patients with mixed pattern tumor are good examples of this.

The significance of nuclear grade as a criterion for classifying DCIS into high and low nuclear grade of malignancy has been emphasized by Bellamy et al.[3] In fact, in their series, they found that invasive recurrence followed only high nuclear grade DCIS, regardless of the histologic pattern.

There also have been attempts to evaluate other features seen in DCIS, but the significance of those features has not been demonstrated. Lagios et al.[6] reported an intermediate grade for DCIS, in which recurrence was intermediate between high and low grade. Recently, Lennington et al.[8] focused their attention on assessing necrosis because they found a group of noncomedo DCIS in which necrosis was present and whose average size and nuclear grade appear to fall into an intermediate position between comedo and noncomedo without necrosis DCIS, suggesting that this could be an intermediate grade lesion. The same attempts to find an intermediate group led a group of European pathologists to classify DCIS into groups of well, intermediate, and poorly differentiated, based on nuclear and architectural features[18]; however, these designations seem inadequate to us because the DCIS does not form differentiated ductal structures, as would be expected, at least for the well differentiated type.

The general analysis of our results confirmed the heterogeneity of DCIS for most of the markers used and allowed the division of DCIS into three groups: high, intermediate, and low grade. The high grade group, which included all patients with comedo and micropapillary types and one patient with mixed type, was characterized by high nuclear grade and usually the presence of prominent necrosis. In 4 of 18 patients with high grade DCIS, necrosis was not seen, but the high nuclear grade allowed them to be included in this group. In addition, the patients with high grade DCIS had tumors with immunoreactivity to c-*erb*B-2 and aneuploidy much more frequently than did patients in the other groups (including patients without necrosis), and the difference of expression is statistically significant. These findings are in accordance with those reported in the literature, which indicate that using the architectural classification of DCIS shows that comedocarcinomas are most frequently aneuploid and c-*erb*B-2 positive.[10–12] When we used the architectural, approach we found the same correlation. The low grade group was characterized by a high frequency of low nuclear grade and absence of necrosis. In this group, c-*erb*B-2 expression was rare, and euploidy was seen more frequently than was aneuploidy. A third and intermediate group of DCIS, characterized by the presence of necrosis not associated with comedo features, also was identified. Most of these patients had an intermediate nuclear grade. The expression of c-*erb*B-2 and ER in this group was similar to that observed in the low grade group, but ploidy was similar to that observed in the high grade group because 80% of them were aneuploid.

We found a high frequency of positivity to ER (63.9%) and PR (65.7%) in our patients with DCIS. The positivity did not correlate with the histologic subtype, but a near significant association between high grade DCIS and lack of ER was identified ($P = 0.06$). However, this lack of ER expression becomes significant only when we compare high grade DCIS with intermediate and low grade together ($P = 0.02$); in fact, only 43.7% of high grade tumors express ER, whereas 80% of low and intermediate grade DCIS tumors were positive for ER. We used a monoclonal antibody 1D5 with microwave antigen retrieval to assess ER in DCIS. Because this antibody correlates well with H222 and biochemical ER assessment in invasive breast carcinomas,[19] the results can be compared with other series that used H222 to assess ER in DCIS. Although we have shown some correlation between ER expression and histologic grading, Poller et al.[13] found a correlation between ER positivity and small cells with a noncomedo architectural pattern in DCIS.

We found immunohistochemical expression of p53 in 36.8% of our patients; p53 expression did not discriminate among the high, intermediate, or low grade groups of DCIS, and p53 was not expressed differently among the histologic subtypes of DCIS. However, in a comparison of our patients with low grade DCIS with those with intermediate and those with high grade DCIS, we found 16.7% of positivity in the first group and 46.2% in the second. These results, although not significant ($P = 0.08$), seem to support those of Poller et al.,[14] who showed an increased frequency of p53 positivity in DCIS with more aggressive histologic features, which in their series were considered to be large cells and the presence of necrosis.

The current study provides additional evidence indicating the morphologic and biologic heterogeneity of DCIS of the breast. The three groups proposed according to the histologic assessment of grade of malignancy (high, intermediate, and low grade) have a good and significant correlation with some of the additional parameters studied, namely immunoreactivity for c-*erb*B-2 and ploidy. In our study, the expression of c-*erb*B-2 and aneuploidy (namely the pattern IV) constitute additional and significant criteria of high grade of malignancy. The need to include an intermediate group of malignancy in DCIS is supported by our findings because tumors of this group express some of the characteristics of those of the high grade group (necrosis and aneuploidy) and some of the low grade group (rare c-*erb*B-2 expression and common ER expression). Whatever the morphometric and immunohistochemical markers identify, clinically useful subsets of DCIS tumors have not been established; doing so will require long-term prospective studies of patients having conservative care.

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