Biologic Markers in Ductal Carcinoma In Situ and Concurrent Infiltrating Carcinoma

A Comparison of Eight Contemporary Grading Systems

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Key Words: Ductal carcinoma in situ; Grading; Invasive ductal carcinoma; Estrogen receptor; Progesterone receptor; E-cadherin; MIB1; p27; Vimentin; c-erbB-2; Architecture; Nuclear grade

Abstract

The relevance of 8 contemporary classification and grading systems for ductal carcinoma in situ (DCIS) of the breast was examined in 100 tumors by comparing DCIS grade with grade of the concurrent infiltrating ductal carcinoma (IDC). Besides tumor size and nodal status, the immunohistochemical parameters in both lesions were compared, including estrogen receptor, progesterone receptor, c-erbB-2 protein, E-cadherin, vimentin, Ki-67 (MIB1), and p27. Nuclear grading of DCIS alone or in combination with architectural pattern and necrosis showed the best correlation with grade of the invasive component. There also was a positive correlation between every biologic marker expressed in DCIS and in the concurrent IDC, supporting a clonal relationship. Biologic markers varied between the different grades of DCIS. DCIS is heterogeneous, and the progression of DCIS to IDC may be from low-grade DCIS to low-grade IDC and high-grade DCIS to high-grade IDC. This concept is different from the conventional model held for intraepithelial neoplasia in the cervix, vulva, vagina, and skin, in which there is increasing severity of in situ atypia (dysplasia) before the development of stromal invasion.

The incidence of ductal carcinoma in situ (DCIS) of the breast seems to be increasing markedly, particularly with the advent of mammography. In the United States, during the 20-year period 1973-1992, age-adjusted DCIS incidence rates increased more than 6-fold, from 2.3 to 15.8 per 100,000 female population; the major increase occurred during the 1983-1992 period.1 Dramatic increases were observed in women 50 years old or older, lower increases in the 40- to 49-year-old group, and only modest increases in the 30- to 39-year-old group.

It has long been recognized that in some patients, DCIS will progress to invasive carcinoma if it is untreated or treated inadequately.2 When the disease is recurrent, almost half of the cases show invasive histologic features.3 Currently, all cases of DCIS are managed in the same manner, as though they share the same or approximately equivalent potential for malignant progression, and all forms of therapy involve surgical excision and/or sterilization with radiation.4 It is increasingly evident that DCIS is not a homogeneous disease. DCIS shows different grades of malignant potential. Some subtypes of DCIS are more likely to recur, and some express biologic markers that are recognized as markers of poor prognostic outcome in invasive ductal carcinomas.4,5 Traditionally, the classification of DCIS has been based on architectural patterns that divide into comedo, solid, cribriform, and micropapillary subtypes.5 Lagios et al10 were among the first to incorporate nuclear grade and necrosis with cytoarchitecture for the classification of DCIS. Subsequent classifications have been based primarily on nuclear cytology,11 cytologic and architectural features including the polarity of cells,12 cytologic features and the presence or absence of necrosis (the Van Nuys classification),4 cytologic and architectural features and necrosis in combination13 or separately,9 and the extent of necrosis as the sole criterion.14
The existence of several classifications, more appropriately called grading systems, for DCIS inevitably leads to confusion, particularly for the lesser experienced. The present study was designed to assess the applicability and relevance of 8 major grading systems for DCIS. One method of assessing the clinical relevance of any grading system would be to correlate the various subtypes with tumor recurrence and progression. However, this information was not available at the time of reporting, as follow-up was too short. An alternative method is the correlation of each subtype with the biologic phenotype, as some phenotypes are recognized to be associated with more aggressive behavior. Importantly, the correlation of DCIS grade with the grade and biologic phenotype of the concurrent infiltrating ductal carcinoma (IDC) provides an indication of the relevance of the grading system. In the present study, each of the histologic features, namely, architectural pattern, nuclear grade, and the presence or absence of necrosis, was assessed separately and correlated with the biologic parameters.

Materials and Methods

We retrospectively examined 379 consecutive breast tumor biopsy specimens accessioned at the Division of Tissue Pathology, Institute of Medical and Veterinary Science, Adelaide, Australia, from January 1993 through December 1995. These represented screen-detected as well as symptomatic lesions and mastectomy specimens. Any case of lumpectomy followed by mastectomy or wider excision was considered as one specimen, and cases where primary diagnostic or follow-up biopsies were examined by another laboratory were excluded. Specimens that did not contain DCIS, those with lobular carcinoma in situ or infiltrating lobular carcinoma, and those that contained insufficient material for immunohistochemical studies were also excluded. The final number of cases showing DCIS with associated IDC was 100.

Clinical Data

The clinical notes of all cases were reviewed for the following data: patient’s age and method of detection of breast lesion. In addition, the tumor size as measured on the slide, the total number of axillary nodes dissected, and the number of nodes with metastasis were obtained from the pathology report.

Immunohistochemical Studies

Representative blocks from all tumors were subjected to immunostaining for the following markers using a streptavidin-biotin peroxidase technique as previously described: estrogen receptor (ER; 1:100 dilution; clone 1D5, DAKO, Santa Barbara, CA), progesterone receptor (PR; 1:60 dilution; clone KD 68, Abbott, North Chicago, IL), growth fraction as assessed with Ki-67 (1:500 dilution; clone MIB1, Immunotech, Westbrook, ME), c-erbB-2 (1:600 dilution; polyclonal, DAKO), E-cadherin (1:100 dilution; clone HEC-D-1, Zymed Laboratories, San Francisco, CA), p27 (1:1,000 dilution; monoclonal, Transduction Laboratories, Lexington, KY), and vimentin (1:1,000 dilution; clone V9, DAKO). All primary antibodies were used following microwave-stimulated antigen retrieval according to protocols previously described. Target Retrieval Solution (DAKO) was used as the retrieval solution. Immunostaining in DCIS and infiltrating carcinoma components were evaluated independently by 3 of us (R.T.S., R.W.H., and S.V.). ER, PR, Ki-67, and p27 are nuclear antigens; c-erb-B2 and vimentin show membrane and cytoplasmic localization, respectively; and E-cadherin shows both membranous and cytoplasmic localization. Only membranous c-erb-B2 and E-cadherin staining was considered positive and was assessed as follows: 0, no staining; 1, weak staining; 2, intermediate staining; and 3, strong staining. Vimentin staining was assessed as follows: 0, no staining; 1, 0% to 50% of tumor cells positive; and 3, more than 50% of tumors cells positive. The method of assessing Ki-67 counts has been described in detail. A similar method was adopted for p27. ER and PR scores were based on the number of positively staining tumor cells as follows: 0, no staining; 1, less than 10%; 2, 11% to 25%; 3, 26% to 50%; 4, 51% to 75%; and 5, more than 75%.

Classification and Grading of DCIS and Infiltrating Carcinoma

All tumors were examined and graded independently by 4 pathologists (A.S.-Y.L., R.W.H., S.V., and C.S.). In the event of disagreement, the grading was assigned after discussion and by consensus. This was attained by reviewing the sections in a multiheaded microscope in conjunction with written criteria for grading. It was necessary in a few instances to compare the sections with previously graded examples in which opinions were unanimous. In these 8 cases, the majority opinion was recorded.

DCIS was graded according to the Nottingham modification of the Bloom and Richardson system. DCIS was graded according to the following 8 major systems. The reader is referred to the original publications for details.

1. The Bellamy classification was based on predominant architectural pattern as comedo, solid, cribriform-micropapillary, or papillary types and further qualified into low (grade 1) and high (grades 2 and 3 combined) grade according to nuclear grade.

2. The Holland classification was based primarily on cytonuclear differentiation and, secondarily, on...
architectural differentiation or cellular polarization. Well-differentiated DCIS showed monomorphic, regularly spaced nuclei with fine chromatin, inconspicuous nucleoli, and few mitoses. Pronounced polarization produced cribriform, micropapillary, and clinging patterns. Necrosis was uncommon. Poorly differentiated DCIS showed pleomorphic, irregularly spaced nuclei with coarse, clumped chromatin, prominent nucleoli, and frequent mitoses. There was no architectural differentiation; the pattern was solid or pseudocribriform. Necrosis was usually present. Intermediate DCIS showed cytologic features in between, always with polarization around intercellular spaces but not as pronounced as the well-differentiated group.

3. The Lagios classification\(^1\) evaluated nuclear grade, cytologic architecture, and necrosis. Subtypes I and II were described as generally “characterized by large cells with prominent nuclear pleomorphism, dyskaryosis, and comedo necrosis.” The 2 subtypes differed only in cytologic architecture—solid in type I and papillary and/or cribriform in type II. Type III displayed large but more uniform cells with modest nuclear pleomorphism and dyskaryosis, a prominent cribriform pattern, and rare small punctate foci of coagulative necrosis or none at all. Subtype IV lesions contained uniform small cells arranged in a cribriform or micropapillary pattern without necrosis. Lagios\(^2\) subsequently developed a revised classification with a 3-tiered system. Low-grade lesions had a nuclear diameter of 1 to 1.5 times that of RBCs, diffuse chromatin, no nucleoli, less than 1 mitosis per 10 high-power fields (hpf), and no necrosis; intermediate-grade lesions had a nuclear diameter of 1 to 2 times that of RBCs, coarse chromatin, infrequent nucleoli, 1 to 2 mitoses per 10 hpf, and 1+ necrosis; and high-grade lesions had a nuclear diameter of more than 2 times that of RBCs, vesicular nuclei with coarse chromatin, 1 or more nucleoli, 2 or more mitoses per 10 hpf, and 3+ necrosis.

4. Leal et al\(^9\) graded according to nuclear morphologic features. Grade 1 lesions had nuclei 1 to 2 times the size of nuclei in adjacent normal breast duct epithelium, were monomorphous, and had inconspicuous nucleoli. Grade 2 nuclei showed moderate pleomorphism, were 3 to 4 times the size of nuclei of adjacent breast duct epithelium, and often had evident nucleoli. Grade 3 nuclei showed marked nuclear pleomorphism, frequently contained multiple conspicuous nucleoli, and were 5 or more times the size of nuclei in benign duct epithelium. While architecture also was assessed, there was considerable overlap of nuclear grades between each architectural type.

5. The Van Nuys classification\(^4\) divided DCIS into cytologic high grade and non–high grade; the latter was further subdivided on the basis of necrosis or no necrosis.

6. DCIS also was graded by pattern alone into comedo, solid, cribriform-micropapillary, papillary, and mixed types. Comedo DCIS was defined as a solid arrangement of tumor cells with more than 50% of the cross-sectional area of the duct showing necrosis. When necrosis was less than 50% of cross-sectional area, the lesion was designated solid DCIS with necrosis. Cribriform-micropapillary DCIS was defined as ducts filled with evenly spaced cells arranged around punched-out, rounded, intercellular spaces to produce a cribriform pattern; whereas the micropapillary pattern was composed of multiple micropapillae around the circumference of the duct that projected into the lumen with indiscernible fibrovascular cores, sometimes forming “rigid” bridges to produce a cribriform pattern. Combinations of cribriform and micropapillary patterns were common. Papillary DCIS was composed of uniform-appearing cells arranged as papillary projections with true fibrovascular cores. This pattern was very uncommon in this series and was incorporated with the cribriform-micropapillary group. The tumors were classified according to the predominant pattern (>50%). If a predominant pattern was not observed, the lesion was classified as mixed.

7. Cytologic grading was based on nuclear morphologic features. Grade 1 nuclei were monotonous and 1.5 to 2.0 times the size of RBCs or duct epithelial cell nuclei. Chromatin was finely dispersed, nucleoli were only occasional, and mitotic figures uncommon. Grade 2 nuclei were intermediate in appearance between grades 1 and 3. Grade 3 nuclei were markedly pleomorphic and more than 2.5 times the size of RBCs or duct epithelial cell nuclei. Nuclei usually were vesicular with coarse chromatin, often prominent and multiple nucleoli, and frequent mitoses.\(^2\)

8. Necrosis was defined as the presence of ghost cells and karyorrhectic debris. Necrosis had to be centrally located in the duct lumen to be considered significant necrosis, and punctate or focal necrosis was discounted.\(^2\)

Statistical Analysis

The association between DCIS according to the different grading systems and concurrent IDC was analyzed by using kappa statistics.\(^2\) The correlation between the biologic parameters in DCIS and the concurrent IDC was analyzed by using the chi-square test (statistical significance level was set at <.05) and kappa statistics. Each grading...
system for DCIS was correlated with each biologic parameter studied using the Fisher exact test.

Results

One hundred cases of DCIS had concurrent IDC. Figure 1 shows the mean values for each of the biologic parameters studied in DCIS and corresponding IDC for each of the grading systems used. All immunohistochemical parameters showed significant correlation between the DCIS and the concurrent IDC component (P < .0001; chi-square test). The levels of correlation were studied further by using kappa statistics. Table 1 shows DCIS grades according to the different systems correlated with grades of concurrent IDC using kappa statistics. The highest kappa values were obtained with nuclear grading alone (kappa = 0.387) and the Bellamy system (kappa = 0.399). The Holland and Lagios systems produced kappa values of 0.324 and 0.275, respectively, whereas all other methods of grading of DCIS produced poor kappa values. By Fisher test, only the Van Nuys grading system showed significant correlation with tumor size (P = .01), and nuclear grading showed significant correlation with lymph node status (P = .03) and tumor size (P = .06). Table 2 shows DCIS grades according to the different systems correlated with the greatest number of grading systems for DCIS.

Discussion

The widespread use of screening mammography has resulted in the increasing diagnosis of DCIS, which now represents as many as one quarter of breast cancer diagnoses in many institutions. DCIS has been largely regarded as a homogeneous entity, and because of its potential to progress to invasive carcinoma, has been treated mostly in a standard manner by mastectomy. Since it became apparent that after adequate excision of DCIS invasive cancer develops in only a minority of patients, alternative forms of management have been offered. Pathologists have for some time realized that DCIS is not a homogeneous disease, but there are still no agreed-on criteria to predict the tumors that predispose to subsequent recurrence or invasive cancer.

In an attempt to stratify DCIS according to biologic behavior, several classifications or, more correctly, grading systems have been proposed. The traditional system has been based primarily on architecture, but newer systems take into account nuclear grade, as well as the amount of necrosis present. The proliferation of pathologic grading systems for DCIS is reminiscent of the situation with malignant lymphoma classifications in the late 1970s and prompted an international consensus conference on DCIS in 1997. While an agreed-on classification for DCIS was not a result, conference participants recognized that DCIS should be stratified primarily by nuclear grade. Necrosis was identified as a feature that modified the risk associated with nuclear grade, and cell polarization tended to be a feature of DCIS of lower grade lesions. Architectural pattern alone was not a consistent association of nuclear grade and did not stratify DCIS satisfactorily. Importantly, the conference provided a consensus definition of nuclear grade and necrosis, the criteria for which were used in the present study. It also emphasized the need to identify the biologic and molecular markers that predict recurrence, progression to invasion, and response to therapy as an important goal of future studies.

The conventional method of verifying the relevance of grading systems is through clinical follow-up for recurrence or progression of the disease, ie, the recurrence of DCIS or the development of IDC. However, it could be argued that the recurrent lesion, which can manifest after a variable time interval, might not necessarily be related to the original neoplastic clone, or it may represent subclones of the original. Therefore, there is merit in comparing the morphologic features and biologic profile of the preinvasive tumor with those of the concurrent IDC as we have done. A biologic similarity of both lesions would support their clonal relationship, and comparison of the grade of DCIS and IDC would validate the relevance of the DCIS grading system. Our findings indicate that the Bellamy (kappa = 0.399), the Holland (kappa = 0.324), and nuclear grade (kappa = 0.387) showed the strongest correlation with the grade of the concurrent IDC. Importantly, our results also indicate that all biologic characteristics of DCIS, including the proliferative index, showed moderate to substantial correlation with those of the concurrent IDC. These findings reveal that when DCIS is associated with a concurrent IDC, it is likely to be one of similar grade and similar biologic characteristics, suggesting that they represent neoplastic cells of the same clonal population. It has been shown that tumor grade does not change between primary and recurrent breast carcinoma, as there was concordance of grade between the primary tumor and axillary lymph node metastases and with subsequent locally recurrent and metastatic lesions in 115 patients with infiltrating ductal carcinoma of the breast. Our observation that the tumor cells are phenotypically different between different grades of DCIS lends credence to the concept that DCIS is a heterologous disease.

Several studies have shown positive correlations between a variety of biologic markers with different DCIS
Mean scores for biologic markers in 100 cases of ductal carcinoma in situ (DCIS) and infiltrating ductal carcinoma (IDC) according to 8 grading systems for DCIS. The vertical axes represent percentage counts or scores according to the biologic marker assessed, as given in the text. MIB1 and p27 counts were scored as a percentage of positive tumor cells. ER and PR scores were 0, no staining; 2, <25%; 3, 26%-50%; 4, 51%-75%; 5, >75%. c-erb-B2 scores were 0, no staining; 1, weak; 2, intermediate; 3, strong. Vimentin scores were 0, no staining; 1, <0% of tumor cells positive; 2, >10% of tumor cells positive. MIB1D, mean MIB1 count in DCIS; MIB1, mean MIB1 count in IDC; ERD, mean c-erb-B2 score in DCIS; ER, mean c-erb-B2 score in IDC; PRD, mean progesterone receptor score in DCIS; PR, mean progesterone receptor score in IDC; VimD, mean vimentin score in DCIS; Vim, mean vimentin score in IDC; p27D, mean p27 count in DCIS; p27, mean p27 count in IDC.
grades. By using an enzyme-linked immunoabsorbent assay in 151 cases, Poller et al. found a positive correlation between Nottingham grading of DCIS and ER status. Others, using immunohistochemical staining, also found a positive correlation or showed a trend between DCIS grade and ER status. We found positive correlations between ER and each grading system except the Leal. Similar to our findings, Bobrow et al. and Bose et al. have demonstrated positive correlations between grades of DCIS and PR expression. Strong correlations have been shown between various grading systems of DCIS with c-erb-B2 over-expression; membranous staining was found mostly in comedo-type high-nuclear-grade DCIS. While a trend was evident in our study, it did not attain statistical significance. Together, these findings lend support to the concept that DCIS is heterogeneous but related to the concurrent invasive cancer. There is also some molecular and cytogenetic evidence that DCIS is the direct precursor of IDC. Lukas et al., in a recent study of 31 cases with evidence of both DCIS and IDC, showed that there were p53 mutations in 6 cases. By using single-strand conformational polymorphism analysis, they demonstrated that in each case there was an identical mutation in the DCIS and the concurrent IDC, indicating a clonal relationship between the two.

Of all the biologic parameters assessed, the proliferative index, as assessed with MIB1 immunostaining, gave the strongest correlation with all grading systems for DCIS. There also was strong correlation of the proliferative index with various grading systems of DCIS with c-erb-B2 over-expression; membranous staining was found mostly in comedo-type high-nuclear-grade DCIS. While a trend was evident in our study, it did not attain statistical significance.

### Table 1

**kappa Statistics for Biological Markers in DCIS and Concurrent IDC**

<table>
<thead>
<tr>
<th>Biological Marker</th>
<th>kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIB1, DCIS/MIB1, IDC</td>
<td>0.465, moderate</td>
</tr>
<tr>
<td>ER, DCIS/ER, IDC</td>
<td>0.782, substantial</td>
</tr>
<tr>
<td>PR, DCIS/PR, IDC</td>
<td>0.723, substantial</td>
</tr>
<tr>
<td>c-erbB2, DCIS/c-erb-B2, IDC</td>
<td>0.748, substantial</td>
</tr>
<tr>
<td>Vimentin, DCIS/vimentin, IDC</td>
<td>0.606, moderate</td>
</tr>
<tr>
<td>E-cadherin, DCIS/E-cadherin, IDC</td>
<td>0.585, moderate</td>
</tr>
<tr>
<td>p27, DCIS/p27, IDC</td>
<td>0.770, substantial</td>
</tr>
</tbody>
</table>

DCIS, ductal carcinoma in situ; ER, estrogen receptor; IDC, infiltrating ductal carcinoma; PR, progesterone receptor.

* A chi-square value of \( P < .0001 \) was found for every parameter.

### Table 2

**Grading for Ductal Carcinoma In Situ According to 8 Contemporary Systems Correlated With Grade of the Concurrent Infiltrating Ductal Carcinoma (IDC) and Using kappa Statistics**

<table>
<thead>
<tr>
<th>IDC Grade</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bellamy</td>
<td></td>
<td></td>
<td></td>
<td>0.399 (fair)</td>
</tr>
<tr>
<td>Low</td>
<td>26*</td>
<td>—</td>
<td>13†</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>13†</td>
<td>—</td>
<td>49†</td>
<td></td>
</tr>
<tr>
<td>Holland</td>
<td></td>
<td></td>
<td></td>
<td>0.324 (fair)</td>
</tr>
<tr>
<td>Well differentiated</td>
<td>16</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Intermediate differentiated</td>
<td>13</td>
<td>12</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>7</td>
<td>15</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Lagios</td>
<td></td>
<td></td>
<td></td>
<td>0.275 (fair)</td>
</tr>
<tr>
<td>Type I</td>
<td>13</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Type II</td>
<td>16</td>
<td>9</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Type III</td>
<td>7</td>
<td>15</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Leal</td>
<td></td>
<td></td>
<td></td>
<td>0.179 (slight)</td>
</tr>
<tr>
<td>Low grade</td>
<td>15</td>
<td>9</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Intermediate grade</td>
<td>17</td>
<td>6</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>High grade</td>
<td>4</td>
<td>14</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Van Nuys</td>
<td></td>
<td></td>
<td></td>
<td>0.210 (slight)</td>
</tr>
<tr>
<td>1</td>
<td>13</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>19</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Nuclear grade</td>
<td>1</td>
<td>20</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>13</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>12</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Necrosis</td>
<td></td>
<td></td>
<td></td>
<td>0.387 (fair)</td>
</tr>
<tr>
<td>Absent</td>
<td>17</td>
<td>21†</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>23</td>
<td>39‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Architectural pattern</td>
<td></td>
<td></td>
<td></td>
<td>0.096 (poor)</td>
</tr>
<tr>
<td>Cribriform-micropapillary</td>
<td>24</td>
<td>19</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Solid</td>
<td>7</td>
<td>6</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Comedo</td>
<td>3</td>
<td>13</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

* Low grade.
† High grade.
‡ Grades 2 and 3 combined.
DCIS and the concurrent invasive cancer. It would be interesting, in follow-up studies, to determine whether this marker provides as useful information as histologic classification and grading to predict the recurrence of DCIS. In an earlier study of infiltrating breast carcinoma, Goldstein and Murphy showed that the proliferative index was a major factor in the prediction of risk of relapse and death from disease.

Progression through the cell cycle is governed by a family of cyclin-dependent kinases (CDKs), which are activated by binding to cyclin proteins regulated by phosphorylation at specific residues and inhibited by the CDK inhibitors. The p27Kip1 gene encodes an inhibitor of CDK activity. Reduced levels of p27 protein have been identified in a number of human cancers, and, in some cases, the reduced levels were associated with an increase in the proliferative fraction of the tumor. In keeping with these findings, we found that p27 protein staining showed a negative correlation with the grade of DCIS and was inversely correlated with MIB1 counts. Thus p27 may be a suitable alternative to Ki-67 counts in the assessment of cell proliferation.

We found that several grading systems (Bellamy, Holland, Lagios, Van Nuys, and nuclear grade alone) revealed a positive correlation between the staining for vimentin in DCIS and in the IDC component, and vimentin staining was seen in high-grade DCIS. Vimentin immunostaining has been found mainly in hormone receptor–negative, high-grade carcinomas, suggesting that vimentin may be a poor prognostic marker. Follow-up studies have shown vimentin expression to be associated with poor prognosis in node-negative ductal carcinomas. Seshadri et al found that although vimentin expression was associated significantly with high-grade tumors, absence of hormone receptors, increased p53 expression, and high proliferative index, it did not seem to influence overall survival and risk of relapse of disease. Vimentin gene expression in breast cancer cell lines seemed to be associated with a hormone-independent and more aggressive subset and tended to be fibroblastoid and more invasive both in vitro and in vivo. In vitro models indicate that overexpression of vimentin-intermediate filaments leads to augmentation of motility and invasiveness. Our findings of vimentin staining in high-grade DCIS and their concurrent high grade IDC adds further support to the contention that the two forms of tumor represent progression from the same clone with associated poor prognostic markers.

Immunostaining for c-erb-B2 revealed a substantial correlation (kappa = 0.748) between the overexpression of this protein in DCIS and the concurrent IDC. c-erb-B2 often is expressed in DCIS and frequently in high-grade lesions. The expression of this protein has been correlated with other poor prognostic variables in DCIS. c-erb-B2 overexpression has been shown to correlate with p53 immunostaining and overexpression, high nuclear grade, and massive necrosis, and it is inversely related to hormone receptor status.

Current grading systems of DCIS use nuclear grade as the most important parameter and differ largely by the incorporation of architectural pattern, as in the Bellamy system, and pattern and necrosis in both the Holland and Lagios systems. The Leal system, which proved to be the least relevant in the present study, used nuclear grading, but the criteria were different from those of other systems. Nuclei of grades 2 and 3 were defined as 3 to 4 times and >2 times the RBC diameter, respectively. These are much larger than the dimensions used in the Van Nuys grading system (1-2 times and >2 times the RBC diameter, respectively) and the consensus conference criteria (2-2.5 times and >2.5 times the size of RBCs or nuclei in benign ductal epithelium, respectively) for grades 2 and 3 DCIS.

We found 2 major drawbacks in the application of systems that use a combination of nuclear grade and architectural pattern. One was the heterogeneity of architectural patterns within the same lesion; the other was the variation of nuclear grade within the same pattern subtype. For example, cribriform DCIS can show low, intermediate, and, less commonly, high nuclear grades. The Van Nuys classification

![Table 3](https://example.com/table3.png)

Contemporary Grading Systems for Ductal Carcinoma In Situ Correlated With Biologic Parameters

<table>
<thead>
<tr>
<th>Grading System</th>
<th>Tumor Size</th>
<th>Lymph Node Status</th>
<th>MIB1</th>
<th>ER</th>
<th>PR</th>
<th>c-erb-B2</th>
<th>Vimentin</th>
<th>E-Cadherin</th>
<th>p27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bellamy</td>
<td>NS</td>
<td>NS</td>
<td>.02</td>
<td>.02</td>
<td>.06</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Holland</td>
<td>NS</td>
<td>NS</td>
<td>.004</td>
<td>.004</td>
<td>.001</td>
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<td>Van Nuys</td>
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<td>NS</td>
<td>.003</td>
<td>.004</td>
<td>.000</td>
<td>NS</td>
<td>.05</td>
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<tr>
<td>Nuclear grade</td>
<td>.06</td>
<td>.03</td>
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<td>NS</td>
<td>.01</td>
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<tr>
<td>Necrosis</td>
<td>Present</td>
<td>NS</td>
<td>.01</td>
<td>NS</td>
<td>.08</td>
<td>NS</td>
<td>NS</td>
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</tr>
<tr>
<td>Architectural pattern</td>
<td>NS</td>
<td>NS</td>
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<td>NS</td>
<td>NS</td>
<td>.003</td>
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ER, estrogen receptor; NS, not significant; PR, progesterone receptor.

* Chi-square or Fisher test, P < .05 considered significant. See “Materials and Methods” for explanations of the grading systems.
avoids this problem by using nuclear grade as the main criterion with further subdivision by the presence or absence of necrosis.

A few studies have examined the applicability and reproducibility of DCIS classifications, and, judging from the outcome of the consensus conference, which failed to advocate any one system, the search for a generally acceptable classification will continue. In this regard, we found that nuclear grading according to the criteria set by the consensus conference produced significant correlations with the largest number of parameters known to be of prognostic relevance in both the concurrent IDCs as well as in the DCIS. Badve et al reported a statistically significant correlation between nuclear grade as defined by the European Pathologists Working Group and recurrence. A significant correlation also was found using the Van Nuys classification, but the authors stated that “further work is necessary to determine whether nuclear grade or necrosis is more appropriate to subdivide the non–high grade cases.” We did not find necrosis alone to be a significant feature. Another study that divided DCIS into 3 grades according to nuclear morphologic features and the presence or absence of cell polarization found poorly differentiated lesions to correlate with occurrence of invasive carcinoma, large DCIS, marked necrosis, and periductal inflammation and fibrosis. A study among 23 pathologists of the European Commission Working Group on Breast Screening Pathology found that the highest kappa statistics (0.35-0.42) were obtained with the Van Nuys system and grading systems with 3 grades. The assessment of cell polarization in addition to nuclear grade neither improved nor worsened consistency of grading.

In epithelial sites such as the cervix, vulva, vagina, and skin, the natural history of dysplasia is conceived as a morphologic and biologic continuum. There is progression over many years from mild atypia (dysplasia) to moderate atypia (dysplasia) to severe atypia (dysplasia) to carcinoma in situ before eventually becoming invasive, ie, a vertical progression. It would seem that this conventional model, ie, epithelial hyperplasia without atypia and atypical ductal hyperplasia progressing to DCIS with increasing ductal in situ dysplasia before the development of stromal invasion, is unlikely to apply to DCIS in the breast. Our findings suggest an alternative mode of progression. The grade and biologic profile of DCIS are correlated significantly with an invasive tumor of corresponding grade and biologic profile, suggesting that in most cases, low-grade DCIS is associated with low-grade invasive carcinoma and high-grade DCIS with high-grade invasive carcinoma, ie, a horizontal progression. It is our impression that intermediate-grade DCIS is heterogeneous, so it is possible that this group represents some cases of DCIS that have progressed from low-grade DCIS and cases that may progress to high-grade DCIS, so progression may not be entirely horizontal in intermediate-grade DCIS. Support for this contention comes from the work of Buerger et al, who studied the chromosomal alterations in DCIS using comparative genomic hybridization. They found that about 50% of 38 DCIS cases displayed 1 of 3 main genetic abnormalities, which was characteristic for each grade of DCIS according to the Holland system. They concluded that high-grade DCIS was genetically different from low- and intermediate-grade DCIS and that the evolution of high-grade DCIS was most likely along a different pathway from other grades. Interestingly, they also showed that in the cases with concurrent IDC, the genetic aberrations were identical in both invasive and noninvasive components. Unfortunately, the authors did not grade the IDC component in their study. Thus, it would seem that many of the prognostic features of breast cancer are already established at the DCIS stage of the neoplastic process as suggested by Gupta et al, who did not examine
biologic parameters but based their concept on morphologic
grounds and clinical follow-up.

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