

Correlation of Nuclear Morphometry with Pathologic Parameters in Ductal Carcinoma *In Situ* of the Breast

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Morphometric features of nuclear perimeter, nuclear area, feret ratio, and feret circle were studied in a series of 64 cases of ductal carcinoma *in situ* (DCIS) of the breast in Singapore women. The results were compared with pathologic parameters of tumor size, nuclear grade, necrosis, cell polarization, and architectural pattern. There was statistically significant correlation between nuclear perimeter and area with all the pathologic parameters, with the strongest association observed for nuclear grade ($P < .0001$). Higher grade nuclei as assessed histologically were associated with larger nuclear area ($44.14 \mu\text{m}^2$ in low-grade lesions, $47.77 \mu\text{m}^2$ in intermediate-grade lesions, and $72.05 \mu\text{m}^2$ in high-grade lesions) and perimeter ($25.94 \mu\text{m}$ in low-grade nuclei, $27.12 \mu\text{m}$ in intermediate-grade nuclei, and $33.66 \mu\text{m}$ in high-grade nuclei). DCIS lesions with necrosis and absence of polarization also revealed increased nuclear area and perimeter ($P < .05$). Comedo architecture was associated with larger nuclear area and perimeter ($65.97 \mu\text{m}^2$, $31.7 \mu\text{m}$) than the papillary subtype ($42.17 \mu\text{m}^2$, $25.29 \mu\text{m}$), with the mixed morphologic pattern disclosing intermediate values ($54.83 \mu\text{m}^2$, $29.43 \mu\text{m}$). There was direct correlation for tumor size with nuclear area and perimeter ($P < .01$). No similar relationship was found between pathologic parameters and feret ratio or circle, indicating that nuclear roundness or lack of it did not factor as a significant component in the pathologic assessment.

KEY WORDS: Breast, DCIS, Morphometry, Pathologic parameters.

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Ductal carcinoma *in situ* (DCIS) of the breast, generally regarded as the precursor of most invasive breast cancers, has increased dramatically in incidence, due primarily to mammographic screening (1). In Singapore, it was found to comprise 25% of screen-detected breast cancers (2), with 94% of DCIS lesions associated with radiologic calcifications (3). Because it is a heterogeneous lesion, pathological classification schemes that attempt to define its biologic potential in terms of local recurrence and progression to invasive carcinoma have evolved, and debate continues as to the best system to adopt (4).

Interobserver reproducibility studies have also been performed to define the system that allows the greatest concordance in pathologic assessment (5-8), with most advocating nuclear grade as the criterion that achieved the best agreement.

In this paper, we document the correlation of pathologic variables determined from routine macroscopic and microscopic assessment of DCIS lesions with morphometric nuclear features derived quantitatively from image analysis. The aims are to investigate the association between histologically assigned nuclear grade and quantitative nuclear parameters, confirm its reproducibility with an objective imaging system, and define the relationship of other pathologic criteria with the nuclear morphometric findings.

MATERIALS AND METHODS

Patients, Tumors, and Pathological Evaluation

The study population comprised 64 women diagnosed with DCIS of the breast between 1993 and 1999, obtained from the files of the Department of Pathology, Singapore General Hospital. Tissues were fixed in 10% buffered formaldehyde (pH 7.0), embedded in paraffin, sectioned at $4 \mu\text{m}$, and stained with hematoxylin and eosin (H&E). The pathological features analyzed were tumor size, nuclear grade, presence or absence of necrosis or cell

polarization, and architectural subtype. Tumor size was assessed either macroscopically or microscopically. Nuclear grade was assigned in accordance with recommendations of the 1995 NBSBSP publication (9) as low, intermediate, or high. Low-grade nuclei were monomorphic, spherical, and centrally placed with indistinct nucleoli occurring in evenly spaced cells. High-grade nuclei were large, with irregular nuclear contours, coarse chromatin, and prominent nucleoli, observed in pleomorphic, irregularly spaced cells displaying frequent mitoses. Intermediate-grade nuclei straddled in between, with mild to moderate pleomorphism that was less than that seen in high-grade nuclei; however, the cells lacked the monotony of the low-grade lesions. The presence of cell polarization was defined as a radial orientation of the cell apices toward an intercellular lumen (10). Architectural subtypes included comedo, cribriform, papillary, micropapillary, and mixed categories.

Image Cytometry

Nuclear morphometry was performed in a blinded fashion by BBG (without knowledge of the histologic assessment) on representative H&E slides containing lesional tissue, selected for optimum histologic detail. The Kontron Elektronik imaging system, comprising a light microscope (Axiophot 2, Zeiss) with a ZVS-47DE Carl Zeiss camera linked to a computer using KS400 Release 2.0 software, was used. Frozen sections were excluded because of collapse of nuclei (11). For each case, 150 ductal epithelial nuclei were randomly selected from lesional areas at a magnification of 400X. The images were then digitized and the nuclei outlined using a mouse attached to the computer (12). Only nuclei that could be completely outlined were chosen (Fig. 1). Morphometric parameters studied were nuclear area and perimeter and feret circle and feret ratio, the latter being a ratio of minimum to maximum feret diameter, with a maximum value of 1 corresponding to a circle. Feret circle, a shape factor, is defined by the formula $(4\pi\text{area})/\text{perimeter}^2$. Both feret circle and feret ratio are measures of ellipticity. Values of these morphometric parameters were generated by the computer.

Statistical Analysis

Statistical analysis was performed using the statistical software GraphPad Prism. Student's *t* test

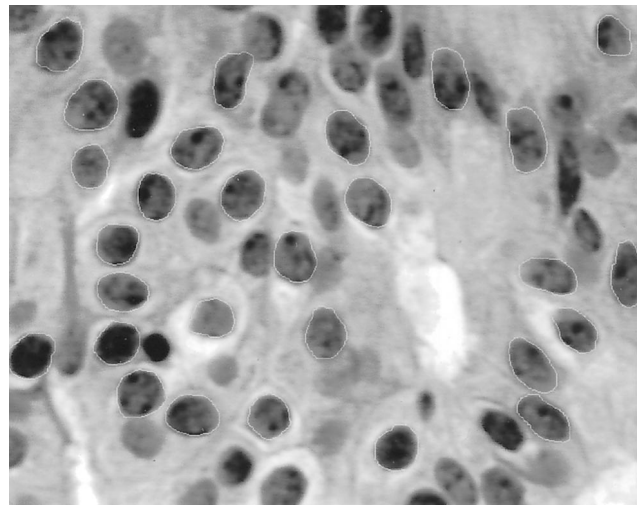


FIGURE 1. Low-grade nuclei of a ductal carcinoma *in situ* lesion digitally outlined using a computer mouse (hematoxylin and eosin staining, original magnification, 400 \times). Cell polarization around a luminal space is noted.

was used to compare means, whereas Pearson's correlation was applied to evaluate relationships between two variables. A statistically significant result was defined as a *P* value of $<.05$.

RESULTS

The patients were aged 33 to 82 years (mean and median, 54 y). Tumor size of all 64 cases ranged from 0.5 to 9 cm, with a mean of 2.2 cm and a median of 1.5 cm. Pathological findings of nuclear grade, necrosis, and cell polarization are detailed in Table 1. Examples of DCIS lesions with low, intermediate, and high nuclear grades are shown in Figure 2. Architectural classification revealed 13 (20%) cases with comedo, 4 (6%) with cribriform, 10 (16%) with papillary, 2 (3%) with micropapillary, 1 (2%) with solid, and 34 (53%) with mixed patterns.

Results of nuclear morphometry are summarized in Table 2. Correlation with histologic pathologic variables revealed that nuclear grade, necrosis, cell polarization, and architectural pattern were significantly associated with nuclear area and perimeter but not with feret ratio or feret circle (Table 3). Owing to insufficient numbers in the other subcategories, only comedo, papillary, and mixed architectural subtypes were analyzed statistically. There was a direct correlation of tumor size with nuclear area and perimeter ($P < .01$; Fig. 3).

TABLE 1. Pathologic Features of Nuclear Grade, Necrosis, and Cell Polarization in 64 Cases of Ductal Carcinoma *In Situ* of Breast

1	Nuclear Grade			Necrosis		Cell Polarization	
	2	3	Absent	Present	Absent	Present	
20 (31)	24 (38)	20 (31)	45 (70)	19 (30)	25 (39)	39 (61)	

Data are given as number (%).

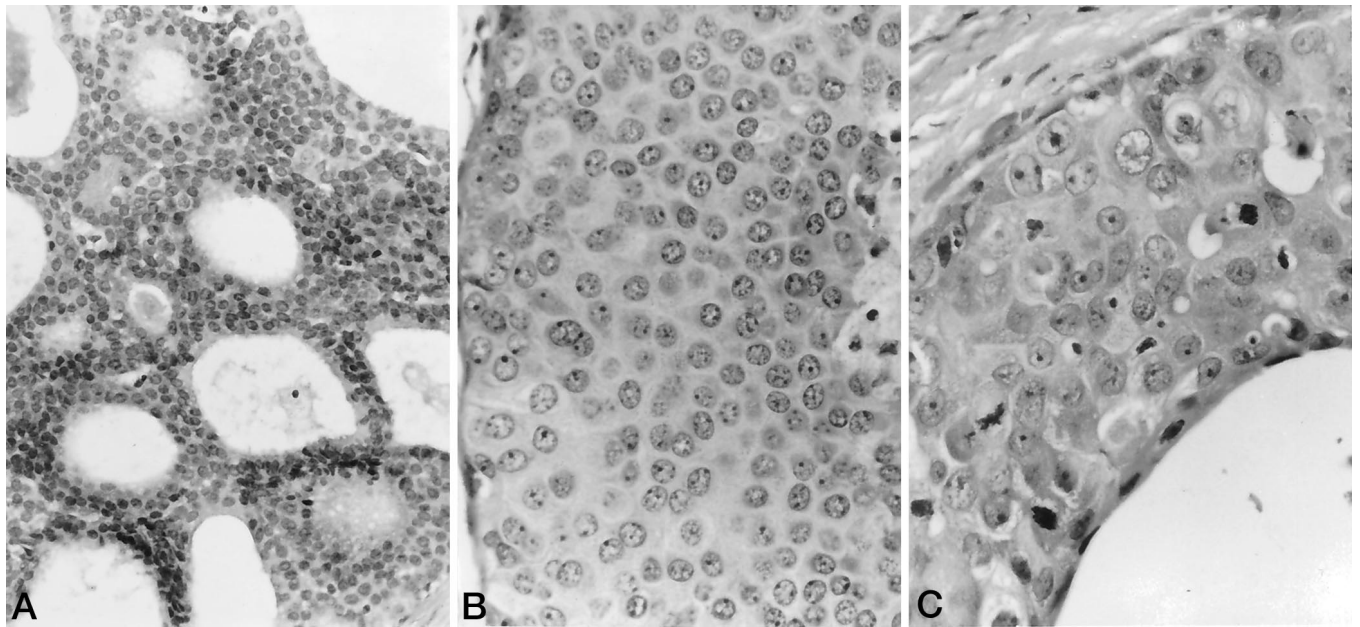


FIGURE 2. A, low-nuclear grade ductal carcinoma *in situ* (DCIS) reveals relatively uniform cells with spherical vesicular nuclei and indistinct nucleoli (hematoxylin and eosin staining; original magnification, 100 \times). B, intermediate-nuclear grade DCIS shows moderate nuclear variation with visible nucleoli. C, high nuclear grade DCIS displays pleomorphic cells with enlarged nuclei, prominent nucleoli, and mitoses. (B and C, hematoxylin and eosin staining; original magnification, 200 \times).

TABLE 2. Nuclear Morphometry in 64 Cases of Ductal Carcinoma *In Situ* of Breast

Parameter	Area (μm^2)	Perimeter (μm)	Feret Ratio	Feret Circle
Minimum	18.54	18.21	0.6214	0.7734
Maximum	103.9	41.59	0.8033	0.9226
Median	49.80	27.76	0.7297	0.8888
Mean	54.29	28.81	0.7278	0.8845
Standard deviation	17.87	4.78	0.0389	0.02682
Standard error	2.233	0.5976	0.004862	0.003353

DISCUSSION

Nuclear morphometry has been shown to be of prognostic utility in invasive breast cancer (13–18). Few studies, however, have reported on its value in DCIS. Bhattacharjee *et al.* (19) found a cutoff difference of 20 μm^2 in mean nuclear area between diseased and normal ducts in the same section to be helpful in discriminating between epitheliosis and DCIS. Pienta *et al.* (20) discovered morphometric determination of nuclear pleomorphism in DCIS to be significantly increased over normal controls; and Poller *et al.* (21) confirmed large nuclear size in *cerbB2* positive DCIS cases.

It has been established that nuclear grade is a strong predictor of recurrence after local excision (22–25) and that DCIS should be classified primarily by nuclear grade (26). Guidelines for nuclear grade assignment are based on several criteria, including the degree of pleomorphism or monotony of the lesional cell population, nuclear–cytoplasmic ratio, nucleolar prominence, and mitotic frequency (9). In addition, size comparison with normal red blood cells or unaffected duct epithelial cell nuclei is ad-

vocated, with low and high nuclear grade DCIS revealing nuclear sizes 1.5 to 2.0 times and more than 2.5 times that of normal red blood cells or unaffected duct epithelial cell nuclei respectively, with intermediate-grade nuclei in between (26). It is interesting that although we graded nuclei intuitively on the subjective impression of nuclear pleomorphism without a deliberate attempt to compare sizes with red blood cells or normal duct epithelium, morphometric assessment reveals that it is the nuclear area, reflecting size, and nuclear perimeter, representing both size and shape (27), but not nuclear roundness and ellipticity (measured by feret ratio and feret circle), that are most strongly correlated. The increasing nuclear size with higher grade DCIS lesions may be explained by the accumulation of abnormal genetic material during carcinogenesis (12).

Though nuclear shape was not a significant factor in this study, it must be noted that there are inherent problems with tracing irregular nuclear contours of malignant cells for image analysis (28). Hence, the lack of association between pathologi-

TABLE 3. Correlation Between Pathologic Parameters and Nuclear Morphometry

Parameter	Mean Nuclear Perimeter (μm)	Mean Nuclear Area (μm^2)	Feret Ratio	Feret Circle
Nuclear grade				
1	25.94	44.14	0.7231	0.8856
2	27.12	47.77	0.7343	0.8873
3	33.66	72.05	0.7269	0.8808
	($P < .0001$)	($P < .0001$)	($P = .6917$)	($P = .7273$)
Necrosis				
Absent	26.21	45.13	0.7189	0.8832
Present	29.99	58.44	0.7327	0.8856
	($P = .0029$)	($P = .0053$)	($P = .1892$)	($P = .7482$)
Cell Polarization				
Present	27.67	48.76	0.7244	0.8846
Absent	30.60	62.91	0.7331	0.8844
	($P = .0153$)	($P = .0015$)	($P = .3876$)	($P = .9767$)
Architectural Pattern				
Papillary	25.29	42.17	0.7121	0.8766
Mixed	29.43	54.83	0.7351	0.8900
Comedo	31.70	65.97	0.7149	0.8692
	($P = .0108$)	($P = .0075$)	($P = .1197$)	($P = .0447$)

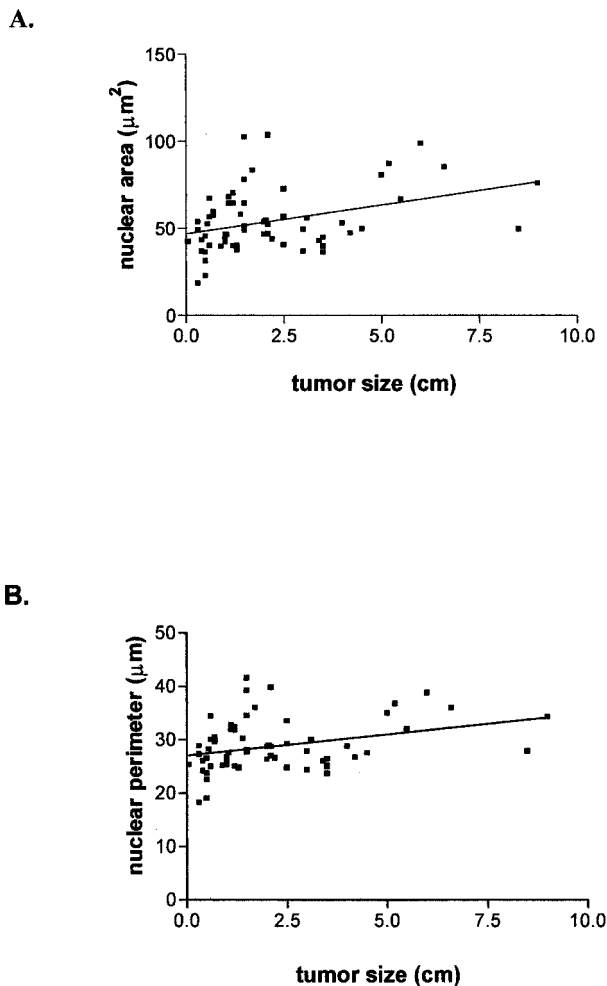


FIGURE 3. Correlation of nuclear area (A) and nuclear perimeter (B) with ductal carcinoma *in situ* tumor size.

cally assigned nuclear grade and the morphometric shape factors may in part be attributed to minor nuclear membrane convolutions and indentations that could have been overlooked during the digiti-

zation procedure. Although the method of processing affects quantitative histopathology, nuclear shrinkage in paraffin-embedded material is less severe (29). Tumor size was also significantly associated with nuclear area and perimeter, supporting the notion that DCIS lesions containing larger nuclei tend to attain greater proportions at presentation.

Likewise, the correlation with necrosis may be partly related to larger tumor size that outstrips its blood supply and partly to greater genetic insult with induction of apoptotic cell death (30). The significant correlation between cell polarization with morphometric nuclear area and perimeter can similarly be explained on the premise that higher grade, larger nuclei lose their ability to polarize or architecturally differentiate around luminal spaces (10).

That DCIS with comedo morphology shows larger nuclei than the papillary subtype is not unexpected. It is noteworthy that lesions with a combination of patterns (mixed category) reveal mean nuclear area and perimeter that are intermediate in value. It has been recommended that nuclear grade of DCIS be based on the highest grade observed (26), despite the heterogeneity that can be encountered. Our study suggests that the mixed architectural pattern may reflect this heterogeneity in nuclear grade and perhaps lend some support to retaining the traditional classification based on morphology, which also has the additional advantage of indicating more extensive disease in the micropapillary subtype (24).

In conclusion, our study shows that the correlation between pathologic parameters of DCIS with nuclear morphometry is strongest for nuclear grade, validating the objectivity and reproducibility of nuclear grade assessment by a quantitative morphometric method. It also reveals that nuclear

grade is correlated with nuclear enlargement than with shape factors, suggesting that nuclear size may be more useful as a criterion for grade assignment.

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