Original Article

Ductal Carcinoma in situ of the Breast. Comparison between the Van Nuys Classification and Nottingham Classification.

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Background: Assessment of the biological malignancy of DCIS is important as a factor related to indications for breast-conserving therapy and subsequent stump recurrence. We assessed the biological malignancy of each grade in the Van Nuys (VN) Classification and the Nottingham (Nott) Classification. **Methods:** The surgical specimens from 24 patients with DCIS were examined. Immunohistochemistry was used to assess the expression of ER, p53, Ki-67 (MIB-1), and Her2/nue in the tumor cell of 24 patients with DCIS. We classified DCIS by VN and Nott systems and compared Grade 3 tumors with the other grades (Grade 1 and 2).

Results: VN Grade 3 (VN3) DCIS had high ER-negative ($P=0.01$) and P53-positive ($P=0.05$) rates, and frequently had a high Ki67 L. I. ($P=0.03$). Three of the 4 specimens highly positive for Her2/nue ($P=0.08$) were VN3. The Nott Grade 3 (Nott3) specimens were all ER-negative ($P=0.03$) and frequently P53-positive ($P=0.04$), and all had a high Ki67 L. I. ($P=0.04$). Similar to VN3 DCIS, 3 of the 4 specimens highly positive for Her2/nue ($P=0.003$) were Nott3. However, 3 specimens that were judged to be nuclear grade (NG) 3 noncomedo type DCIS and VN3 were classified as Nott 1 and 2, not Nott 3. Of these 3 specimens, 2 were ER-negative, 1 was p53 positive, and 2 had a high Ki67 L. I., indicating that non-Nott 3 cancer is not necessarily low-malignant. Conversely, all Nott3 cancers were classified as VN3.

Conclusion: The results suggested that the nuclear-grade-based VN system may be a more reliable classification in terms of grade of malignancy than the histological-type-based Nott systems.

Key Words : ductal carcinoma in situ, Van Nuys Classification, Nottingham Classification, nuclear grade

Introduction

Recent advances in diagnostic techniques have allowed early detection of small primary breast cancers and resulted in high detection rates, particularly of ductal carcinoma in situ (DCIS)¹⁾. DCIS exhibits extensive ductal spreading and often develops multifocally, making it difficult to define

the boundaries of cancer spread by diagnostic imaging²⁾ or determine the boundaries for resection when choosing breast-conserving therapy. Assessment of the biological malignancy of DCIS is important as a factor related to the indications for breast-conserving therapy and subsequent stump recurrence. In this study we immunohistochemically stained surgical specimens from 24 DCIS patients, and assessed the biological malignancy of each grade according to the Van Nuys (VN) Classification³⁾ and the Nottingham (Nott) Classification⁴⁾.

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Patients

The 24 patients (6.5%) who had DCIS among the 370 patients with primary breast cancer who underwent surgery between February 1990 and February 2002 in the Second Department of Surgery, Junten-do University were the subjects of this study. All of the patients were female, and their mean age was 50.7 (30-64). Twenty-one of the 24 patients underwent mastectomy and the other 3 underwent breast-conserving therapy. None have experienced a recurrence (follow-up period up to 10 years, mean 4.4 years).

Classification of DCIS

DCIS was classified by histological patterns into the following types: low-papillary (3 cases), papillary (6 cases), mixed (6 cases), cribriform (2 cases), solid (3 cases), and comedo (4 cases), and by nuclear grade (NG)⁵⁾ into: high NG (NG3), moderate NG (NG2), and low NG (NG1). DCIS was classified as VN3 when the NG of the cancer was high regardless of the presence or absence of necrosis, VN2 when the NG was moderate or low and necrosis was present, and VN1 when NG was moderate or low and there was no necrosis; and according to the Nott Classification into pure comedo DCIS (Nott3), noncomedo DCIS with necrosis (Nott2), and noncomedo DCIS without necrosis (Nott1).

Methods

HE-stained sections of DCIS were examined for histological pattern, NG, and necrosis under a light microscope. Four-micrometer paraffin sections were prepared from formalin fixed, stored specimens and used for immunohistochemical staining. After deparaffinization, sections were treated with 0.01 mol/L citrate buffer (pH 6.0) at 121°C and 1 atm for 15 min, blocked with methanol and 3% H₂O₂ for 30 min, and allowed to react at 4°C for 60 min with a 1:50 dilution of anti-ER α (Santa Cruz Bio., sc-787), anti-Ki-67 (IMMUNOTECH, Lot#, 1298), or anti-P53 (DAKO, Cat#, M7001). Sections were treated with 0.01 mol/L of citrate buffer (pH 6.0) at

95°C for 40 min, blocked with 3% H₂O₂ for 10 min, and allowed to react at 4°C for 60 min with a 1:200 dilution of anti-Her2/neu (DAKO, c-erbB-2, Code A0485). A streptavidin-biotin system (DAKO) was used to detect the bound antibody.

Tissue was examined for ER staining under a light microscope and recorded as positive if more than 20% of the nuclei in the tumor cell had stained, and P53 staining was recorded as positive if any of the tumor cell nuclei had stained. The degree of nuclear staining for Ki-67, an indicator of proliferative activity, was assessed in terms of labeling index (L. I.), that is, the percentage of 1000 nuclei that had positive: over 10% was recorded as highly positive and under 10% as low positive. The degree of Her2/neu staining was classified according to the Hercep Test Atlas (DAKO) as 1+, 2+, or 3+.

The chi-square test was used to perform the statistical analysis. All statistical tests were two sided.

Results

1) Histological pattern and biological markers.

All comedo type tumors (n=4) were NG3, ER-negative, and had a high Ki67 L. I. Three of the 4 comedo type specimens were P53-positive and highly Her2/neu-positive. By contrast, among the noncomedo types, only 1 papillary, solid, and mixed type specimen each was NG3. The ER-positive rate was higher in the noncomedo type than in the comedo type (P=0.006). The Ki67 L. I. in the noncomedo type cases was significantly lower than in the comedo type cases (P=0.01). The noncomedo types had significantly lower P53 (P=0.007) and Her2/neu (P=0.02) positive rates than the comedo type (Table 1).

2) Expression of ER, P53, Ki67, and Her2 by DCIS according to the Van Nuys Classification.

We analyzed expression of the biological markers according to each classification. VN Grade 1 (VN1) DCIS (n=13) had high ER-positive and P53-negative rates and frequently had a low

Table 1 Architectural pattern and biological markers

	Case	NG			necrosis		ER		P53		Ki67 L.I.*			Her2/neu	
		1	2	3	-	+	-	+	-	+	L.I.	low	high	1+2+	3+
Comedo	4	0	0	4	0	4	4	0	1	3	20.8±8.6	0	4	1	3
noncomedo	20														
low papillary	3	1	2	0	3	0	0	3	2	1	4.6±3.8	3	0	3	0
papillary	6	3	2	1	5	1	2	4	6	0	4.8±3.8	5	1	5	1
mixed	6	2	3	1	2	4	1	3	5	1	7.1±5.9	3	3	6	0
cribriform	2	0	2	0	1	1	1	1	2	0	12.9±12.2	1	1	2	0
solid	3	1	1	1	3	0	2	1	2	1	14.1±8.7	1	2	3	0

* Ki67 L.I.: high positive rate >10%, low positive rate ≤10%

Table 2 Expression of ER, P53, Ki67, and Her2 by DCIS according to the by Van Nuys Classification

		Case	VN class (%)			P value
			1	2	3	
ER	positive	14	9 (64)	4 (29)	1 (7)	0.01
	negative	10	4 (40)	0 (0)	6 (60)	
P53	positive	6	2 (23)	0 (0)	4 (67)	0.05
	negative	18	11 (61)	4 (22)	3 (17)	
Ki67 LI.*	>10%	11	3 (27)	2 (18)	6 (55)	0.03
	≤10%	13	10 (77)	2 (15)	1 (8)	
Her2/nue	3+	4	1 (25)	0 (0)	3 (75)	0.08
	1+,2+	20	12 (60)	4 (20)	4 (20)	

* Ki67 L.I.: high positive rate >10%, low positive rate ≤10%

Table 3 Expression of ER, P53, Ki67, and Her2 by DCIS according to the Nottingham Classification

		case	Nott class (%)			P value
			1	2	3	
ER	positive	14	9 (64)	5 (36)	0 (0)	0.03
	negative	10	5 (50)	1 (10)	4 (40)	
P53	positive	6	2 (33)	1 (17)	3 (50)	0.04
	negative	18	12 (67)	5 (28)	1 (6)	
Ki67 LI*	>10%	11	4 (36)	3 (28)	4 (36)	0.04
	≤10%	13	10 (77)	3 (23)	0 (0)	
Her2/neu	3+	4	1 (25)	0 (0)	3 (75)	0.003
	1+,2+	20	13 (65)	6 (30)	1 (5)	

* Ki67 L.I.: high positive rate >10%, low positive rate ≤10%

Ki67 L. I., and they tended to be low positive for Her2/neu. By contrast, VN3 DCIS (n=7) had high ER-negative and P53-positive rates and frequently had a high Ki67 L. I. Three of the 4 specimens that were highly positive for Her2/neu were classified as VN3 (Table 2).

3) Expression of ER, P53, Ki67, and Her2 by DCIS according to the Nottingham Classification.

Nott Grade 3 (Nott3) DCIS (n=4) clearly differed from Nott1 and Nott2 DCIS in expression of biolog-

ical markers. Nott1 DCIS (n=14) had high ER-positive and P53-negative rates and frequently had a low Ki67 L. I., and similar to VN1 DCIS, Nott1 DCIS frequently stained low-positive for Her2/neu. By contrast, the Nott3 specimens were all ER-negative and frequently P53-positive, and all had a high Ki67 L. I. Similar to VN3 DCIS, 3 of the 4 specimens highly positive for Her2/neu were Nott3 (Table 3). We also analyzed expression of the biological markers according to the parameters

Table 4 Expression of ER, P53, Ki67, and Her2 by DCIS according to the Nuclear grade

			NG (%)			
case			1	2	3	P value
ER	positive	14	6 (43)	7 (50)	1 (7)	0.016
	negative	10	1 (10)	3 (30)	6 (60)	
P53	positive	6	0 (0)	2 (33)	4 (67)	0.042
	negative	18	7 (39)	8 (44)	3 (17)	
Ki67LI*	>10%	11	0 (0)	5 (45)	6 (55)	0.005
	≤10%	13	7 (54)	5 (38)	1 (8)	
Her2/nue	3+	4	1 (25)	0 (0)	3 (75)	0.064
	1+,2+	20	6 (30)	10 (50)	4 (20)	

* Ki67 L.I.: high positive rate >10%, low positive rate ≤10%

* : P< 0.01 * * : P<0.05 * * * : P = 0.06

Fig. 1 Biological markers in 24 specimens of DCIS. Comparison between grade 1+2 and grade 3 tumors according to the Van Nuys and Nottingham Classifications

Judging from the expression of ER, P53, Ki67, and Her2/nue, Grade 3 DCIS has a higher grade of malignancy than Grades 1 and 2 DCIS according to both the VN and Nott. classification.

in each classification : NG, histological pattern, and necrosis. NG3 DCIS had a high ER-negative rate, was frequently P53-positive, and frequently had a high Ki67 L. I. There were significant inter-grade differences in expression of the biological markers except Her2/neu (Table 4). As described above (Table 1), comparison between histological patterns showed significant differences in the expression of the biological markers between comedo- and noncomedo-type DCIS (ER, $P=0.006$; P53, $P=0.007$; Ki67 L. I., $P=0.01$; and Her2/neu, $P=0.02$). However, no significant differences were detected between the presence or absence of necrosis and

any of the biological markers (ER, $P=N. S.$; P53, $P=N. S.$; Ki67 L. I., $P=N. S.$; and Her2/neu, $P=N. S.$).

4) Biological markers in the 24 patients with DCIS.

Fig. 1 shows that based on expression of ER, P53, Ki67, and Her2/neu, Grade 3 DCIS has higher malignancy than Grades 1 and 2 DCIS according to both the VN and Nott classifications.

However, 3 specimens that were judged to be nuclear grade (NG) 3 noncomedo-type DCIS and VN3 were not classified as Nott 3. The 3 patients who had these DCIS had a tumor that was his-

Table 5 Cases that were NG3 and the noncomedo type

Case	Architecture	Necrosis	NG	VN	Nott	ER	P53	Ki67 L.I.	Her2
1.	solid	-	3	3	1	-	-	high	1+
2.	papillary	+	3	3	2	+	-	low	2+
3.	mixed	+	3	3	2	-	+	high	1+

tologically solid, papillary, and mixed, respectively (Table 5).

Discussion

Several studies have immunohistologically assessed the biological malignancy of primary breast cancers. Poorly-differentiated DCIS and comedo-type DCIS are very often negative for ER and PgR⁶⁾. Patients with high expression of p53 have a high rate of recurrence in the ipsilateral breast after breast-conserving therapy⁷⁾. The Her2/neu gene is amplified in a high percentage of comedo-type DCIS⁸⁾, and serves as an indicator of the degree of malignancy⁹⁾. Histologically it has been reported that the comedo type has high ER- and PgR-negative rates, a high proliferation index, and high Her2/neu and p53 expression rates^{10,11)}, and that the presence of residual comedo-type DCIS increases the recurrence rate after breast-conserving therapy^{11,12)}. Many such studies have found that the results of immunostaining for biological markers provide a good predictor of local recurrence after breast-conserving therapy^{11,13)}. In this study, we found differences in Ki67 expression according to the histological pattern of DCIS: the comedo-type had the highest cell proliferative activity, while the noncomedo types, i. e., low-papillary type, papillary type, mixed type, cribriform type, and solid type, had increasingly higher Ki 67 L. I. values in that order (Table 1). Together with a study that compared patients whose tumor had a low Ki67 L. I. and showed that patients whose tumor had a high Ki67 L. I. tended to experience local recurrence sooner following breast-sparing therapy¹⁴⁾, the results of the present study support

the report that cell proliferative activity varies with histologic type and that persistence of the highly proliferative comedo type increases the recurrence rate after breast conserving therapy^{11,12)}.

Since Grade 3 DCIS in both classification systems is important when considering the degree of malignancy, we compared expression of the biological markers in Grade 3 DCIS. Immunostaining in these 3 patients (Table 5) showed that cancers included highly malignant ones: 2 cancers were ER-negative; 1 cancer was p53-positive; and 2 cancers had a high Ki67 L. I. Conversely, all Nott3 cancers were found classified as VN3. These results indicate that Nott3 does not include all highly malignant cancers. In other words, in DCIS presenting as heterogeneous histological types, the dispersion of judgments due to histological classification may lead to failure to accurately reflect the degree of malignancy. Therefore, the results suggest that there is little variation in NG in the nuclear-grade-based VN system¹⁵⁾ and that it tends to reflect the degree of malignancy more accurately.

Nuclear grade, a parameter of the VN classification, is useful for assessing the degree of malignancy¹⁶⁾ and predicting the outcome³⁾. We also observed significant differences in expression of the biological markers between the NG1+2 and NG3 cases (Table 4). We often hesitate to judge the histological pattern of DCIS lesion that are heterogeneous, and being heterogeneous may also affect the nuclear grade. Elston et al.¹⁷⁾ have pointed out that the assessment of nuclear grade varies with the pathologist, and there are still no international standards for nuclear grades. On the other hand, Peter Bethwaite et al. have found that interobserver

ver agreement was poorest when the histological scheme was used, and they pointed out that the Van Nuys classification scheme is easy to apply, even to small areas of carcinoma, and resulted in acceptable interobserver agreement between reporting pathologists compared with the modified cytonuclear grading scheme of Holland et al.¹⁸⁾ However, we do not consider it difficult to assess the degree of malignancy based on certain standards without encountering hard-to-assess cancers such as the mixed-type DCIS with various histological patterns.

Conclusion

The results of this study suggested that the nuclear grade-based VN classification may be a more reliable system in terms of grade of malignancy than the histological-type-based Nott system. However, it is necessary to be going to increase the case in the future and to examine it because it is few.

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