ORIGINAL

Thirty percent of ductal carcinoma in situ of the breast in Japan is extremely low-grade ER(+)/HER2(-) type without comedo necrosis

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Abstract : Background Overdiagnosis in mammography (MMG) is a problem. Combination of MMG and ultrasonography for breast cancer screening may increase overdiagnosis. Most cases of overdiagnosis are low-grade ductal carcinoma in situ (LGD), but no reports have focused on them. Materials and methods We immunostained 169 ductal carcinoma in situ (DCIS) cases for ER, PgR, HER2 and Ki67 and classified them into 4 subtypes : ER(+)/HER2(-), ER(+)/HER2(+), ER(-)/HER2(-) and ER(-)/HER2(+). The Ki67 index was used to evaluate the grade of malignancy and examined for correlations with each ER/HER2 subtype and the nuclear grade (NG), with/without comedo necrosis. Results The Ki67 index correlated significantly with NG, both with/without comedo necrosis, and reliably evaluated the grade of malignancy. The index for ER(+)/HER2(-) (n=117, 69.2%) was 7.45±7.10, which was significantly lower than for each of the other types. The index was 5.71±6.94 for ER(+)/HER2(-) without comedo necrosis (n=52, 30.8%), which was significantly lower than with comedo necrosis. This was considered LGD, characterized by absence of microcalcification in MMG and either presence of a solid mass or cystic lesion or absence of hypoechoic areas in ultrasound. Conclusion InJapan, ER(+)/HER2(-) without comedo necrosis accounts for about 30% of DCIS and is LGD. This may be being overdiagnosed. J. Med. Invest. 63 : 192-198, August, 2016

Keywords **:** *low grade DCIS, Ki67 index, ER positive and HER2 negative, comedo-necrosis, mammography*

BACKGROUND

Screening mammography (MMG) is the only diagnostic method that has been proven—by numerous randomized studies and meta-analyses of those studies—to be effective in reducing the mortality rate by breast cancer screening (1, 2). However, in recent years, the usefulness of MMG screening has been called into question (3, 4). For example, it was reported that, even when early cancers were detected by screening MMG, subsequent follow-up studies found that advanced cancers were only marginally reduced. It was concluded that "screening is having, at best, only a small effect on the rate of death from breast cancer" (5). On the other hand, it has been argued that the balance between the benefits and harms of screening is important (2, 6-8). In the 2009 screening mammography guidelines of the US Preventive Services Task Force (USPSTF), the recommendation for breast cancer screening of women aged 40~49 years has been changed from grade B to grade C (9). The reason is that, for that age group, comprehensive evaluation of the benefit (reduction of mortality) and harms (radiation exposure, pain, anxiety, overdiagnosis, and false-negative and false-positive results) showed that the net benefit was small. One of those harms, i.e., overdiagnosis, is defined as "some screening-detected cancers may never progress to be symptomatic in the absence of screening, and some women might die from another cause before the cancer becomes evident" (6).

The introduction of MMG to breast cancer screening brought about a sharp increase in the detection of ductal carcinoma in situ (DCIS). However, invasive ductal carcinoma (IDC) accounts for about 14~50% of DCIS (10, 11), and it can be thought that much of the abovementioned overdiagnosis in breast cancer screening is due to diagnosis of harmful DCIS that does not lead to IDC. Meta-analysis regarding overdiagnosis reportedly showed overdiagnosis of about 11% to 19% in the subject group undergoing screening MMG (6). In Japan, consideration is now being given to combined use of both MMG and ultrasonography for breast cancer screening (7), raising concern that it might lead to even more overdiagnosis.

DCIS is a diverse group of diseases, and it will be necessary to identify (low-grade) DCIS (LGD) in order to reduce overdiagnosis in screening. There have been a few reports regarding assessment of the degree of malignancy of DCIS based on the molecular biology characteristics (12, 13), but there have been no reports that focused on LGD. Moreover, there is no consensus regarding judgment criteria for DCIS based on the ER, PgR and HER2 immunostaining commonly used in clinical medicine, and evaluation of malignancy in DCIS by immunostaining has not been established. Accordingly, we investigated whether it would be possible

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to focus on LGD and apply our experience-based findings for the pathological and immunohistological biomarkers of DCIS in order to identify DCIS having a strong possibility of becoming LGD.

In order to identify image findings characteristic of LGD, we also performed a retrospective re-examination of MMG and ultrasound images of DCIS.

MATERIALS AND METHODS

The subjects of the study were 169 Japanese women who were treated at Tokushima Breast Care Clinic between April 1998 and September 2010. Cases of male breast cancer, bilateral breast cancer and cases of micro-invasion were excluded. Tissue specimens from all the cases were subjected to HE staining and immunostaining for ER, PgR, HER2 and Ki67, and a physician specializing in mammary gland pathology performed the pathological diagnosis and assessed the immunostaining results. The antibodies used for the immunostaining were 1D5 (Dako) (dilution : 1 : 50 ; pretreatment : boiling (pH 9.0, 40 min)) for ER ; PgR 636 (Dako) (dilution : 1 : 800 ; pretreatment : boiling (pH 9.0, 40 min)) for PgR ; HercepTest™ (Dako) for HER2 ; and MIB-1™ (Dako) (dilution : 1 : 50 ; pretreatment : autoclaving) for Ki67. The staining method was the same as the usual method used for invasive cancer. For ER and PgR, the specimen was judged to be positive when ≥ 1% of the cancer cells were stained positively. HER2 staining was judged by the usual method, using scores from 0 to 3+, and scores of 2+ and 3+ were considered positive. Ki67 was judged by using a counting grid to inspect at least 100 tumor cells and then calculating the percentage of positively stained cells to obtain the staining rate. Specimens were classified into four subtypes based on the expression of ER and HER2 : ER(+)/HER2(-), ER(+)/HER2(+), ER(-)/HER2(-) and ER(-)/HER2(+).This study was approved by the Ethics Committee of the University of Tokushima.

This study focused on identifying LGD. The Ki67 index was used to judge the grade of malignancy, and in order to confirm the reliability of that, we investigated the relationship between the index and the nuclear grade (NG), with/without comedo necrosis. Next, we examined the relationships of each ER/HER2 subtype and the NG, with/without comedo necrosis, with the grade of malignancy. Then we examined the relationships of each subtype to the MMG and ultrasound imaging findings. The MMG findings were classified as microcalcification (MC) yes/no, while high-density shadows were classified as a tumor shadow, focal asymmetric density (FAD), distortion or no findings (NF). The ultrasound imaging findings were classified in accordance with the Guidelines for Breast Ultrasound-Management and Diagnosis, 3rd Edition (revised ; edited by the Japan Association of Breast and Thyroid Sonology). That is, mass or non-mass lesions were classified as a solid mass, cystic lesion (cystic or mixed), hypoechoic area, duct-dilatation or NF, while high-echo spots suspected of being MC were classified as MC yes or MC no.

Statistical analysis

The χ^2 or Fisher's exact test was used for comparisons between categorical variables. Differences in mean values between subgroups were tested with the Mann-Whitney *U* test. Multiple group comparisons were performed using the Kruskal-Wallis test followed by the Steel-Dwass *post-hoc* test. Differences were assessed with a 2-sided test and considered significant at the $P < 0.05$ level.

RESULTS

1. Patient background

The patients ranged in age from 29 to 93 y (mean : 57.5 y ; median : 58 y), with a distribution of 32.0% aged ≤ 49 y and 68.0% aged ≥ 50 y. The rates of NG 1, 2 and 3 were 28.4%, 58.0% and 13.6%, while the rates of with comedo necrosis and without comedo necrosis were 65.1% and 34.9%. The positive and negative rates were 82.8% and 17.2% for ER, 74.6% and 25.4% for PgR, and 23.1% and 76.9% for HER2 (Table 1). Based on imaging findings of category 3 and higher, 147 (87.0%) patients had both MMG and ultrasonic findings, one patient had only MMG findings and 19 (11.2%) patients had only ultrasonic findings (dates were not shown). The breakdown of the findings showed, for the MMG findings, 92 (54.4%) patients with MC yes, while for the high density shadows, 38 (22.5%) patients had a tumor shadow, 28 (16.6%) patients had FAD, 3 (1.8%) patients had distortion and 100 (59.2%) patients had NF. In the ultrasonic findings, for mass or non-mass lesions, 60 (35.5%) patients had a solid mass, 12 (7.1%) patients had a cystic lesion, 69 (40.8%) patients had a hypoechoic area, 6 (3.6%) patients had duct dilatation, and 22 (13.0%) patients had NF. High-echo spots suspected of being MC were classified as 'yes' for 78 (46.2%) patients (Table 2).

2. Relationship between Ki67 index and NG, with/without comedo necrosis

Using 15% as the cut-off for the Ki67 index, we classified specimens into low and high groups and investigated the relationship between the Ki67 index and NG, with/without comedo necrosis. The results showed a statistically significant correlation between the Ki67 index low/high and NG, with/without comedo necrosis (Table 3).

3. Relationship between Ki67 index and NG

Detailed examination of the relationship between the Ki67 index and NG was performed, and the Ki67 indexes with NG 1, NG 2 and NG 3 were 8.38±8.76, 8.82±8.65 and 25.17±18.26. The Ki67 index with NG 3 was significantly larger than with the other NG groups ($p < 0.0001$). The difference between NG 1 and NG 2 was not significant (Fig. 1).

4. Ki67 indexes with each subtype, with/without comedo necrosis

The number of patients with each subtype were 117 (69.2%) with ER(+)/HER2(-), 23 (13.6%) with ER(+)/HER2(+), 13 (7.7%) with ER(-)/HER2(-) and 16 (9.5%) with ER(-)/HER2(+) (Table 1). The respective Ki67 indexes were 7.45±7.10, 15.08±12.28, 21.31±21.48 and 21.81±15.95 (mean±SD). The Ki67 index with ER(+)/HER2(-) was significantly lower than with each of the other types. In regard to with/without comedo necrosis, the numbers of ER(+)/HER2(-) type patients with necrosis and without necrosis were both about 50%, but for each of the other types almost all of the patients were 'with necrosis' (Fig. 2). These results indicate that the ER(+)/HER2(-) type is clearly different from the other three types.

5. Ki67 indexes for the ER(+)/HER2(-) type, with/without comedo necrosis

We investigated the Ki67 indexes with the ER(+)/HER2(-) type, with/without comedo necrosis. The Ki67 index with comedo necrosis (n=65, 38.5%) was 8.85±6.96, while without comedo necrosis (n=52, 30.8%) it was 5.71±6.94. The Ki67 index was significantly lower in the case of without comedo necrosis ($p < 0.01$). Also, the distribution of the Ki67 indexes showed a single peak in the case of without comedo necrosis, whereas it showed two peaks in the case of with comedo necrosis (Fig. 3).

Based on these results, we predicted that the ER(+)/HER2(-) type without comedo necrosis is clearly LGD compared with the other types. Hereinafter, in this paper, we defined the ER(+)/HER2(-) type without comedo necrosis as LGD.

Table 1. Various clinicopathological factors in patients with DCIS

		N	(%)
Case No	Total	169	(100.0)
Age (y)	Median, range	58, 29-93	
	≥ 49	54	(32.0)
	≤ 50	115	(68.0)
Nuclear grade	1	48	(28.4)
	2	98	(58.0)
	3	23	(13.6)
Comedo necrosis	Without	59	(34.9)
	With	110	(65.1)
ER	Negative	29	(17.2)
	Positive	140	(82.8)
PgR	Negative	43	(25.4)
	Positive	126	(74.6)
HER2	Negative	130	(76.9)
	Positive	39	(23.1)
Subtype	ER(+)HER2(-)	117	(69.2)
	ER(+)HER2(+)	23	(13.6)
	ER(-)HER2(-)	13	(7.7)
	ER(-)HER2(+)	16	(9.5)
Ki-67	Low (< 15%)	126	(74.6)
	High (≥ 15%)	43	(25.4)
ER(+)HER2(-) with/without comedo necrosis	Yes	52	(30.8)
	No	117	(69.2)

DCIS : ductal carcinoma in situ ; MC : microcalcification ; ER : estrogen receptor ; PgR : progesterone receptor

Table 2. Relationship between Ki67 index, nuclear grade and comedo necrosis in patients with DCIS

	N (%)	Nuclear grade (NG)				Comedo necrosis		
		1 (%)	2 (%)	3 (%)	*P*	Without (%)	With (%)	*P*
Total	169	48 (28.4)	98 (58.0)	23 (13.6)		59 (34.9)	110 (65.1)	
Ki67 index								
Low (< 15%)	126 (74.6)	39 (81.3)	81 (82.7)	6 (26.1)	< 0.0001	53 (89.8)	73 (66.4)	0.0008
High (≥ 15%)	43 (25.4)	9 (18.8)	17 (17.3)	17 (73.9)		6 (10.2)	37 (33.6)	

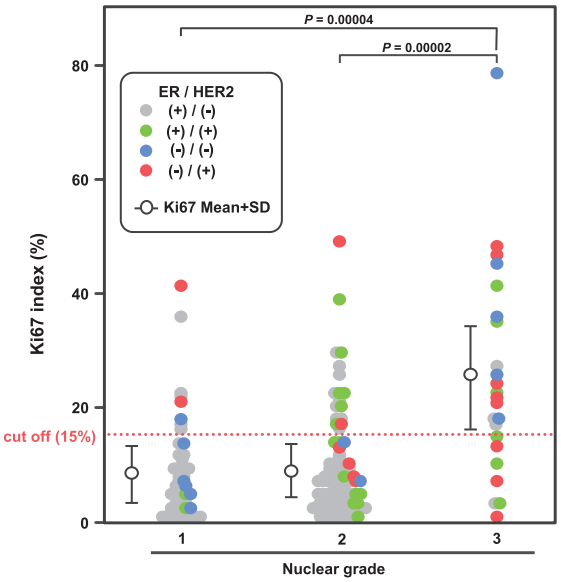
DCIS : ductal carcinoma in situ

Table 3. Relationship between subtypes of DCIS and findings of mammography or ultrasonography

	Subtype	N	MC			High-density shadow				
			No (%)	Yes (%)	*P*	Tumor shadow (%)	FAD (%)	Distortion (%)	No findings (%)	*P*
Mammography	Total	169	77 (45.6)	92 (54.4)		38 (22.5)	28 (16.6)	3 (1.8)	100 (59.2)	
	ER(+)/HER2(-)	117	66 (56.4)	51 (43.6)	< 0.0001	36 (30.8)	19 (16.2)	2 (1.7)	60 (51.3)	< 0.0001
	ER(+)/HER2(+)	23	1 (4.3)	22 (95.7)		2 (8.7)	0 (0.0)	1 (4.3)	20 (87.0)	
	ER(-)/HER2(-)	13	7 (53.8)	6 (46.2)		0 (0.0)	6 (46.2)	0 (0.0)	7 (3.8)	
	ER(-)/HER2(+)	16	3 (18.8)	13 (81.3)		0 (0.0)	3 (18.8)	0 (0.0)	13 (81.3)	

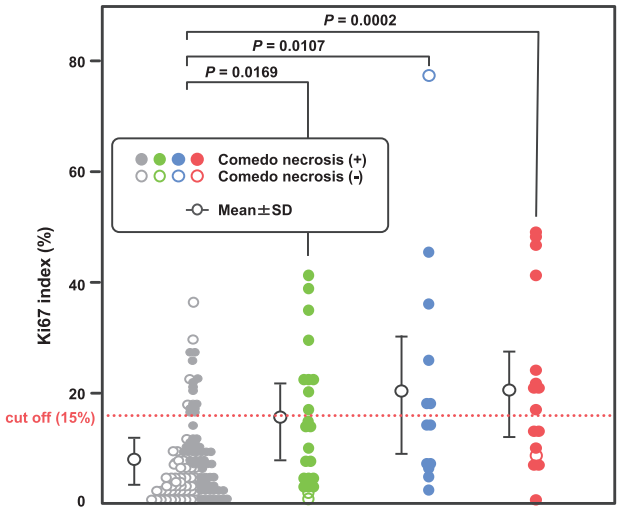
	Subtype	N	Mass or non-mass lesion						High-echo spots (MC)		
			Solid mass (%)	Cystic lesion (%)	Hypo-echoic area (%)	Duct-dilatation (%)	No findings (%)	*P*	No (%)	Yes (%)	*P*
Ultrasonography	Total	169	60 (35.5)	12 (7.1)	69 (40.8)	6 (3.6)	22 (13.0)		91 (53.8)	78 (46.2)	
	ER(+)/HER2(-)	117	51 (43.6)	12 (10.3)	41 (35.0)	3 (2.6)	10 (8.5)	0.0018	74 (63.2)	43 (36.8)	< 0.0001
	ER(+)/HER2(+)	23	4 (17.4)	0 (0.0)	12 (52.2)	0 (0.0)	7 (30.4)		1 (4.3)	22 (95.7)	
	ER(-)/HER2(-)	13	4 (30.8)	0 (0.0)	7 (53.8)	1 (7.7)	1 (7.7)		10 (76.9)	3 (23.1)	
	ER(-)/HER2(+)	16	1 (6.3)	0 (0.0)	9 (56.3)	2 (12.5)	4 (25.0)		6 (37.5)	10 (62.5)	

The χ^2 or Fisher's exact test was used to determine the associations between categorical variations.
DCIS : ductal carcinoma in situ ; MC : microcalcification
ER : estrogen receptor ; HER2 : human epidermal growth factor type 2 ; FAD : focal asymmetric density



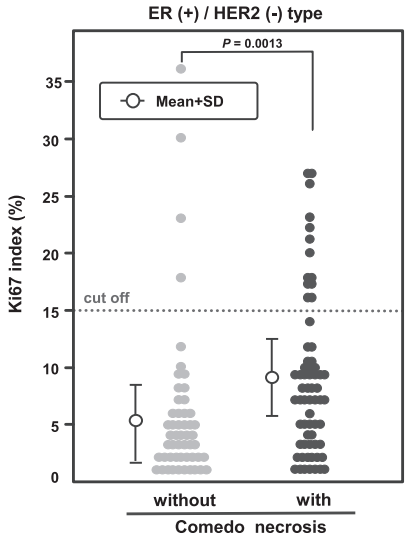
Nuclear grade	1	2	3	total
ER(+) / HER2(-)	38 (32.5%)	75 (64.1%)	4 (3.4%)	117
ER(+) / HER2(+)	2 (8.7)	15 (65.2)	6 (26.1)	23
ER(-) / HER2(-)	6 (46.2)	2 (15.4)	5 (38.5)	13
ER(-) / HER2(-)	2 (12.5)	6 (37.5)	8 (50.0)	16

Figure 1. Relationship between the Ki67 index and the nuclear grade (NG) in the total cases
The cases were classified into subtypes based on the results of immunostaining for expression of ER and HER2. The horizontal bar shows the mean values for each group. The P values show the results of application of the Kruskal-Wallis test ($P=8.31\times10^{-6}$), followed by the Steel-Dwass test.
ER : estrogen receptor ; HER2 : human epidermal growth factor type 2



Mean±SD(%)	7.45±7.10	15.08±12.28	21.31±21.48	21.81±15.95
n	117	23	13	16
Without comedo	52	1	1	1
With comedo	65	22	12	15

Figure 2. The Ki67 indexes for each subtype classified by the expression of ER and HER2
With/without comedo necrosis are shown for each subtype. The horizontal bar shows the mean values for each group. The P values show the results of application of the Kruskal-Wallis test ($P=3.01\times10^{-6}$), followed by the Steel-Dwass test.
ER : estrogen receptor ; HER2 : human epidermal growth factor type 2



Comedo necrosis	without	with
mean±SD(%)	5.71±6.94	8.85±6.96
n	52	65

Figure 3. Relationship between the Ki67 indexes and each ER(+)/HER2(-) type, with/without comedo necrosis
The horizontal bar shows the mean values for each group. The P values show the results of application of the Mann-Whitney test.
ER : estrogen receptor ; HER2 : human epidermal growth factor type 2

6. Imaging findings with each subtype

The imaging findings were compared among the subtypes. In the MMG findings, detection of MC differed significantly among the subtypes ($p<0.0001$). In particular, more than half of the ER(+)/HER2(-) type cases had no MC, whereas almost all of the HER2(+) cases had MC. In the density findings 100 (59.2%) of the 169 patients had no findings. Thirty-six (94.7%) of 38 patients showing a tumor shadow were the ER(+)/HER2(-) type. On the other hand, 19 (67.8%) of 28 patients showing findings of FAD were the ER(+)/HER2(-) type. Next, we compared the ultrasound findings among the subtypes. For mass or non-mass lesions, the 117 ER(+)/HER2(-) type patients showed incidences of 43.6% for solid masses, 10.3% for cystic lesions and 35% for hypoechoic areas, whereas the other types were clearly different in that they showed an incidence of 50% or more for hypoechoic areas. Also, 51 (85%) of 60 patients with findings of a solid mass were the ER(+)/HER2(-) type, while all 12 patients with findings of a cystic lesion were also the ER(+)/HER2(-) type. Moreover, high-echo spots suspected of being MC were not detected in more than half of the ER(+)/HER2(-) type patients, but they were detected in most of the HER2(+) patients. There was thus a clear, significant difference between the subtypes ($p<0.0001$) (Table 3). Moreover, the imaging findings characteristic of LGD were the absence of MC in the MMG findings, and, in the ultrasound findings, either the presence of a solid mass or cystic lesion, or the absence of hypoechoic areas (Table 4).

DISCUSSION

Most overdiagnosis by breast cancer screening is thought to be DCIS. DCIS is said to comprise about 2~5% of symptomatic breast carcinomas (6). It is said that, since the introduction of mammography, from 20% to about half of the discovered cancers are DCIS (6).

Table 4. Relationship between ER(+)/HER2(-)comedo necrosis(-) group of DCIS and findings of mammography or ultrasonography

	ER(+)/HER2(-) Without comedo necrosis	N	MC			Density				
			No (%)	Yes (%)	*P*	Tumor shadow (%)	FAD (%)	Distortion (%)	No findings (%)	*P*
Mammography	Total	169	77 (45.6)	92 (54.4)		38 (22.5)	28 (16.6)	3 (1.8)	100 (59.2)	
	Yes	52	42 (80.8)	10 (19.2)	< 0.0001	23 (44.2)	9 (17.3)	1 (1.9)	19 (36.5)	< 0.0001
	No	110	35 (29.9)	82 (70.1)		15 (12.8)	19 (16.2)	2 (1.7)	81 (69.2)	

	ER(+)HER2(-) Without comedo necrosis	N	Mass or non-mass lesion						High-echo spots (MC)		
			Solid mass (%)	Cystic lesion (%)	Hypo-echoic area (%)	Duct-dilatation (%)	No findings (%)	*P*	No (%)	Yes (%)	*P*
Ultrasonography	Total	169	60 (35.5)	12 (7.1)	69 (40.8)	6 (3.6)	22 (13.0)		91 (53.8)	78 (46.2)	
	Yes	52	31 (59.6)	8 (15.4)	10 (19.2)	1 (1.9)	2 (3.8)	< 0.0001	46 (88.5)	6 (11.5)	< 0.0001
	No	117	29 (24.8)	4 (3.4)	59 (50.4)	5 (4.3)	20 (17.1)		55 (47.0)	72 (61.5)	

The χ^2 or Fisher's exact test was used to determine the associations between categorical variations.
DCIS : ductal carcinoma in situ ; MC : microcalcification
ER : estrogen receptor ; HER2 : human epidermal growth factor type 2 ; FAD : focal asymmetric density

However, DCIS is a heterogeneous disease population, and the grade of the malignancy must be taken into consideration (14-16).

In this study, we used the Ki67 index to judge the grade of malignancy. The diagnostic value of the Ki67 index for grading the malignancy of DCIS has been controversial (17). However, we think that we confirmed the reliability of the Ki67 index for that evaluation because we were able to demonstrate a significant correlation between it and NG, with/without comedo necrosis. First, we investigated the grade of DCIS on the basis of the pathological findings. The results showed no difference between NG 1 and NG 2, while only NG 3 showed a high grade. Also, both NG 1 and NG 2, with comedo necrosis, showed a high grade of malignancy. All cases of NG 3 had necrosis. Therefore, pathologically, both NG 1 and NG 2, without comedo necrosis, showed a statistically significant low grade of malignancy. Various studies investigated the risk of recurrence following breast-conserving treatment of DCIS and reported that there was no difference between low-grade and intermediate-grade malignancy (18) ; that NG was a better predictor of recurrence than comedo necrosis (19) ; and that NG was the most important factor in regard to recurrence of DCIS (20). Although comedo necrosis has less effect than NG, it is considered to be a weak risk factor (21). It was also reported that, in cases of high NG, the presence or absence of comedo necrosis was not a risk factor (22). The above findings were in general agreement with our present results.

We next investigated the grade of malignancy in regard to biomarkers. At present, there are still no definitive judgment criteria for the results of immunostaining for ER, PgR and HER2. For our study, we used a criterion of ≥ 1% for ER and PgR immunopositivity, since in recent years this has been used as the criterion for positivity of these markers in invasive carcinoma (23). Meijinen *et al.* also considered any positive staining result to be the criterion for ER positivity in DCIS (24). On the other hand, we judged HER2 staining to be positive when it was 2+ or 3+, in accordance with the criteria of Kerlikowske *et al.* (25). Thus, we did not investigate cases of 2+ by FISH, etc. Our results showed that the ER(+)/HER2(-) type accounted for approximately 70% of all DCIS, that its Ki67 index was significantly lower than for the other types, and that it was a low-grade malignancy. Moreover, the ER(+)/HER2(-) type without comedo necrosis accounted for about 30% of the total cases of DCIS, and its grade of malignancy was significantly lower than that of the ER(+)/HER2(-) type with comedo necrosis. We considered the ER(+)/HER2(-) type without comedo necrosis to be the most harmful population within DCIS, i.e., LDG. Moreover, it was indicated that the ER(+)/HER2(-) type with comedo necrosis might include a mixture of two DCIS types, one with the same characteristics as without comedo necrosis and a second with a slightly higher grade of malignancy.

A biomarker-based study of the risk of local recurrence following breast-conserving treatment of DCIS found that high levels of expression of p16, COX-2 and Ki67 were significant risk factors (25, 26). Moreover, with regard to ER, HER2 and Ki67, ER(-), HER2(+) and Ki67(+) were reported to be risk factors (25). The results of immunohistochemical studies of 314 DCIS patients showed that there were significantly many cases of local recurrence in the higher tumor grade, positive margin status, luminal B, HER2(+) groups. Moreover, the ER(-)/HER2(+) group was the type with the most recurrence, while the luminal A type had fewer cases of recurrence (27). Our findings are in agreement with those results. In addition, HER2(+) and Ki67 were reported to be risk factors that are independent of the nuclear grade and patient age (28).

Next, in our investigation for possible relationships between the imaging findings and the subtypes, we found that more than half of ER(+)/HER2(-) type cases were MC no, whereas almost all HER2(+) type cases were MC yes. Detection of MC was significantly different between these subtypes. Moreover, for the ultrasound findings, the results showed that the ER(+)/HER2(-) type was significantly more common in cases with solid mass or cystic lesion. Also, the imaging findings characteristic of LGD were the absence of MC in the MMG findings, and, in the ultrasound findings, either the presence of a solid mass or cystic lesion, orthe absence of hypoechoic areas.

The cases we studied included DCIS detected by ultrasound. Approximately 80% of breast cancers detected by screening in Europe and the USA are detected by MMG, and most cases of DCIS were detected by MC in the MMG findings (29). In Japan, on the other hand, ultrasound examinations are becoming more commonly performed on an outpatient basis. In our patient cohort, as well, DCIS was discovered in 19 (11.2%) patients on the basis of only ultrasound findings, which far exceeds the single case that was detected only on the basis of MMG findings. If we consider the fact that, in our present results, the case of MC yes detected by MMG was DCIS with a significantly high degree of malignancy, then it indicates that many cases of low-malignancy DCIS are being detected by ultrasonography in Japan. That can also be surmised from the fact that, in our present cases, the ER(+)/HER2(-) type

comprised 69.2% of the total DCIS cases, which is a clearly higher incidence than the 54.6% rate reported by Meijnen *et al.* for the Netherlands (30). Moreover, the previously noted results of meta-analysis of overdiagnosis showed an overdiagnosis rate of 11% to 19%. On the other hand, our present rate of LGD (that is, cases with high potential for overdiagnosis) was 30.8%. Although those two data cannot be directly compared, we can readily conclude that this incidence of LGD is high, which also suggests that many cases of low-grade DCIS are being detected in Japan. In addition, there is a possibility that cases of LGD are also included in the ER(+)HER2(-) type with comedo necrosis, which permits us to estimate that around half of DCIS cases in Japan are a result of overdiagnosis. Introduction of breast cancer screening that employs ultrasonography is now being considered in Japan (7), but there is concern that this will even further increase detection of LGD. There is also the risk that false-positives will increase. The handling of BI-RADS MMG findings has been changed so that detailed examinations would be necessary only for IDC and high-grade DCIS. It is thought that this change will reduce not only cases of overdiagnosis, but also false-positives and unnecessary biopsies (31). We think that this same sort of reconsideration is also necessary for the handling of ultrasonography findings.

There is also a need to investigate the role of age, but we have not done this here because of the small sample size. Even if DCIS were to develop into IDC, it would take a considerable number of years, and it will be necessary to increase the number of cases and investigate this in the future.

Moreover, recent research has shown that there are harmful cases even among IDC (32). Wells *et al.* reported that 30% of IDC cases are ultra-low risk (33). It will be necessary to identify harmful IDC and elucidate its characteristic imaging findings so that it will be possible to construct an effective screening system for high-risk cases.

CONCLUSION

Our investigation of the grade of DCIS by using the Ki67 index as an indicator elucidated that the ER(+)/HER2(-) type is significantly lower in its degree of malignancy compared with the other types of DCIS. Within that type, cases without comedo necrosis showed significantly lower malignancy than cases with comedo necrosis, and it was considered to be the least malignant form of DCIS (LGD). The imaging findings characteristic of LGD were absence of MC in the MMG findings, and, in the ultrasound findings, either the presence of a solid mass or cystic lesion, or the absence of hypoechoic areas. However, the sample size of this study was small, and it will be necessary to analyze a larger number of cases. There is an urgent need to proceed with this research in order to reduce the harm caused by breast cancer screening.

ABBREVIATIONS

MMG : mammography ; DCIS : ductal carcinoma in situ ; ER : estrogen receptor ; PgR : progesterone receptor ; HER2 : human epidermal growth factor receptor 2 ; NG : nuclear grade ; LGD : low-grade DCIS ; IDC : invasive ductal carcinoma ; MC : microcalcification ; FAD : focal asymmetric density ; PPV : positive prediction value ; NPV : negative prediction value

CONFLICT OF INTERESTS

None of the authors have any conflicts of interest associated with this study.

FINANCIAL INTERESTS

The authors declare that they have no financial interests with this study.

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