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Clinicopathological features of breast cancer without mammographic findings suggesting malignancy

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ABSTRACT

Background: Mammography (MG) is widely used for screening examinations. Dense breast reduces MG screening sensitivity, possibly delaying diagnosis. However, little is known about the characteristics of breast cancers without MG findings indicative of malignancy. Hence, we investigated breast cancer patients with tumors not detected by MG.

Patients and methods: In total, 1758 Japanese patients with breast cancer, undergoing curative surgery between 2012 and 2018 without neo-adjuvant chemotherapy, were retrospectively investigated. Clinicopathological features were compared between patients without (MG-negative) and with (MG-positive) cancer-specific findings on MG. The current study included cases who came to our hospital after experiencing subjective symptoms, or whose tumors were detected by MG and/or US-screening. We reviewed results of both MG and US conducted at our institution.

Results: There were 201 MG-negative cases (11.4%). In patients with invasive disease, multivariate analysis revealed MG-negative patients to have higher breast density on MG ($p < 0.001$). Tumors of MG-negative patients were smaller ($p < 0.001$), showed less lymph node involvement ($p = 0.011$), and were of lower grade ($p = 0.027$). The majority of MG-negative tumors were found by ultrasound screening, being smaller than tumors in patients with subjective symptoms. In the MG-negative group, tumor characteristics such as tumor grade did not differ between those detected by screening versus subjective symptoms.

Conclusion: Most tumors in MG-negative group patients were identified by US screening and the diseases were found at early stages with low malignancy. The usefulness of additional ultrasound with MG-screening might merit further investigations.

1. Introduction

Mammography (MG) is an established means of early detection and diagnosis of breast cancer [1–3]. MG has long been used for screening examinations worldwide, although the starting age and frequency of screening vary among countries, reflecting differences in several factors such as clinical guidelines, medical insurance system coverage and local traditions.

The finding of “dense breast”, defined as background breast tissue with a high density on MG, is a major factor reducing MG screening sensitivity [4,5]. Dense breast can mask a breast cancer, which would thus not be detectable by MG alone, possibly delaying diagnosis. Women with dense breasts might be recommended to undergo additional imaging tests such as 3-D mammography (breast tomosynthesis), breast magnetic resonance imaging (MRI) and ultrasonography (US) [6–8], although these imaging modalities are not consistently available in all regions and countries. Many studies have suggested US to possibly be a good complement to MG, especially for patients with dense breast tissue [5,9]. In the United States, supplemental screening with US is increasingly being

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Abbreviations

The area under the curve AUC
Body mass index BMI
Estrogen receptor ER
Human epidermal growth factor receptor 2 HER2
Mammography MG
Magnetic resonance imaging MRI
Progesterone receptor PgR
The receiver operating characteristic ROC
Ultrasonography US

used in clinical practice [10]. In Japan, where population-based MG is offered every two years starting from 40 years of age, the usefulness of a combination of MG and US was recently demonstrated in a large prospective randomized study [11]. However, additional US is not currently recommended by the government. Offering the MG and US combination throughout the nation, requiring numerous experienced technicians with up-to-date US equipment, is as yet unrealistic. In order to make up for the shortcomings of the MG screening and build a more efficient screening system, we have aimed to ascertain the characteristics of breast cancers that are undetectable on MG.

While limitations of MG for dense breasts and characteristics of breast cancer arising from dense breast tissues have been well documented by numerous studies [12,13], little is known about the clinicopathological features of breast cancers without MG findings indicative of malignancy. Breast cancer diagnosed after negative MG, also referred to as interval breast cancer, is known to have aggressive biological features [14,15]. However, most such tumors develop between screening examinations, rather than being undetectable on MG. As yet, other studies have focused on the diagnostic performance of US in MG-negative cases and revealed improved cancer detection with additional US [16–21]. However, these studies examined screening sensitivity in women with dense breasts and direct comparisons of tumors with/without MG-findings have rarely been conducted.

Hence, to reveal the clinicopathological features of breast cancers undetectable on MG, we retrospectively investigated and compared breast cancer patients according to MG findings.

2. Patients and Methods

2.1. Screening system for breast cancer in Japan

In Japan, biennial MG, in a government-led screening program, is recommended for women age 40 years and older. Meanwhile, working women under 40 have an essentially equal chance of undergoing screening MG, supported by their employers. Women who are not working also have the opportunity to be examined in screening systems offered by their husband's companies. There are also private companies encouraging additional US-screening regardless of recipient age as health promotion services. Therefore, screening-detected tumors in our cohort included those that were found by MG and/or US. Women age 40 years and older can choose between municipality- and company-provided examinations.

2.2. Patients

In total, 2155 breast cancer cases underwent curative surgery between July 2012 and December 2018 at our department. We studied cases for whom all clinical records and MG imaging data were available. We also excluded cases who received neo-adjuvant chemotherapy before surgery. In cases with metachronous ipsilateral breast cancer, only the first disease was investigated. As a consequence, 1758 cases, all Japanese, were retrospectively investigated in the current study. The current study included cases who had initially presented with symptoms, i.e. subjects were not limited to those whose tumors were detected by the screening program.

Employing clinical records, motivations, i.e. the triggers, for visiting the hospital, were also investigated. These data are based on the reasons given by patients for coming to our hospital. Thus, some patients already had symptoms but came to the hospital to participate in a screening program with a referral letter. Moreover, there were patients with masses that they had not palpated themselves, such that the number with "subjective symptoms" might be less than in those with a "palpable" mass, as assessed by clinicians (data shown in the Results section with Fig. 1).

This study was carried out with approval from the ethics committee of Juntendo University Hospital (no.19–289) and the research plan is presented on the homepage of our hospital. All patients were offered the choice to opt-out of the study at any time.

2.3. Imaging assessment

Two-dimensional conventional MG and US were conducted for all patients at our institution. MG assessments were routinely carried out at Juntendo University Hospital by two experienced radiologists. These data were retrospectively collected and analyzed in the current study. When the radiologists examined the MG, breast density was first categorized into Almost entirely fatty, Scattered areas of fibroglandular density, Heterogeneously dense, and Extremely dense, according to the BI-RADS Atlas 5th edition established by the American College of Radiology (available on https://www.acr.org/Clinical-Resources/Reporting-and-Data-Systems/Bi-Rads). Breast densities of the latter two categories were defined as dense breast in the current study. Categorizations of the MG features were based on the Japanese Radiological Society and the Japanese College of Radiology (the 3rd edition, 2014), as follows; Category 1: Negative, Category 2: Benign, Category 3: Probably benign but malignancy cannot be ruled out, Category 4: Possibly malignant, and Category 5: Highly suggestive of malignancy. As to concordance with the BI-RADS assessment categories, the Japanese categorization is used only for imaging assessment, while BI-RADS takes patient management factors into consideration (Table 1). The Japanese categorization is employed at both medical check-up facilities and hospitals performing detailed examinations, such that there is no correspondence with BI-RADS Category 6. The main difference involves Category 3. Category 3 in the Japanese system simply indicates patients requiring additional imaging and/or pathological assessment, while BI-RADS Category 3 is only for clearly benign cases comprehensively assessed with other modalities such as US. Categories 1 and 2 (MG-negative) in the Japanese categorization, the main focus of the current study, basically correspond to the BI-RADS system.

Furthermore, we investigated the US findings in detail. US was pre-surgically conducted for all patients using Aplio 500 (Canon Medical Systems Corporation, Tochigi, Japan). Based on the Japan Association of Breast and Thyroid Sonology (the 3rd edition, 2014), US lesions were first classified as mass or non-mass types. Mass lesions were categorized as solid, intracystic and cyst only types. Non-mass lesions were classified into ductal ectasia, low echoic and architectural distortion types.

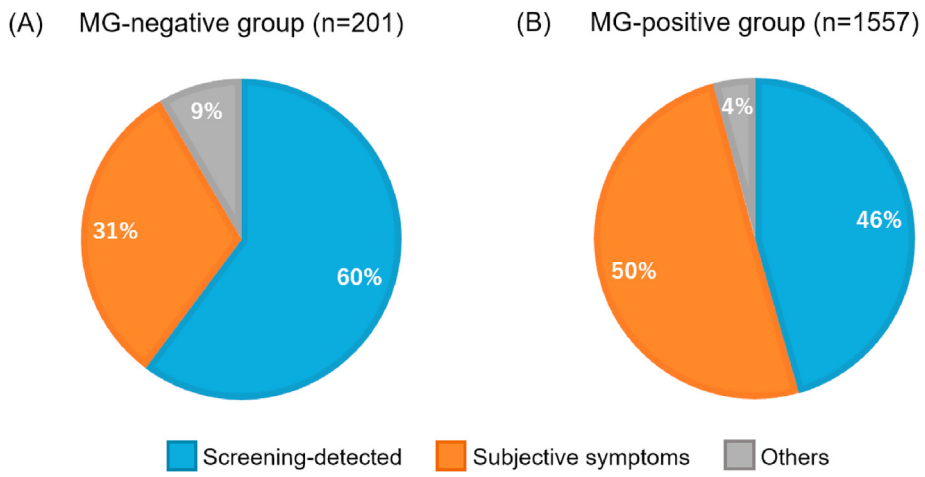

Fig. 1. Motivations for visiting the hospital according to MG categorizationMotivations for visiting the hospital according to MG categorization are shown.
Screening-detected cases in the MG-negative group (A) correspond to US-detected cases since there were no patients who had cancer-specific findings on MG. Meanwhile, screening-detected cases in the MG-positive group (B) include those detected by MG and/or US. "Others", the category represented in grey, includes patients who came to our hospital for follow-up after the initial examination for breast cancer or benign breast diseases.

Table 1
Correspondence between MG categorizations and BI-RADS assessment.

Definition in the current study	Japanese categorization		BI-RADS category	
	Category	Assessment	Assessment	Category
MG-negative	1	Negative	Negative	1
MG-negative	2	Benign	Benign	2
MG-positive	3	Probably benign but malignancy cannot be ruled out	Probably benign	3
MG-positive	4	Possibly malignant	4A: Low suspicion for malignancy	4
MG-positive	4	Possibly malignant	4B: Moderate suspicion for malignancy	4
MG-positive	4	Possibly malignant	4C: High suspicion for malignancy	4
MG-positive	5	Highly suggestive of malignancy	Highly suggestive of malignancy	5
			Known biopsy-proven malignancy	6

2.4. Assessment of pathological factors

Pathological examinations were carried out at Juntendo University Hospital by two experienced pathologists. Tumor grade was judged based on the modified Bloom-Richardson histologic grading system. On immunohistochemistry, estrogen receptor (ER) and progesterone receptor (PgR) statuses were assessed semi-quantitatively and reported as positive when more than 1% of the nuclei of cancer cells showed staining. Human epidermal growth factor receptor 2 (HER2) was judged to be positive when strong staining of the entire cell membrane was observed in >10% of tumor cells or *HER2/neu* gene amplification was confirmed by fluorescence in situ hybridization. For immunohistochemistry of Ki67, mouse monoclonal antibody, clone MIB-1 (Dako, Tokyo, Japan), was used. For the Ki67 labeling index, a hot spot was chosen in one high power field and cells positive for nuclear Ki67 were then semi-quantitatively assessed. All tumor sizes shown as data throughout the current study are based on pathological assessment.

2.5. Statistical analysis

Statistical analyses were performed using JMP 14.2 statistical software (SAS Institute, Inc., Cary, NC, USA). For comparisons of mean values, such as those for age, examinations of unpaired data were carried out employing the two-sided Student t-test. A logistic regression model was constructed in an attempt to discover the factors characterizing MG-negative cases. For continuous variables such as age, body mass index (BMI), tumor size, and the Ki67 labeling index, cut-off values discriminating MG-negative from those categorized as C-3 to C-5 (MG-positive) were determined first, allowing the receiver operating characteristic (ROC) curves to be drawn. Cut-off values for these factors were 53 years for age, 23 for BMI, 16 mm for tumor size and 43% for the Ki67 labeling index, with the respective area under the curve (AUC) values being 0.62, 0.59,

Table 2
Clinicopathological features according to MG categorization (n = 1758).

Variables		MG-negative group	MG-positive group	Univariate			Multivariate		
				OR	95% CI	p value	OR	95% CI	p value
n		201	1557						
Age	>53	63 (31%)	832 (53%)	0.40	0.29–0.54	**<0.001**	0.60	0.41–0.87	**0.007**
	≤53	138 (69%)	725 (47%)						
Menopause	Yes	71 (35%)	849 (55%)	0.45	0.33–0.61	**<0.001**			
	No	126 (63%)	674 (43%)						
	Unknown	4 (2%)	34 (2%)						
BMI	>23	51 (25%)	578 (37%)	0.58	0.41–0.80	**<0.001**	0.87	0.59–1.25	0.451
	≤23	150 (75%)	979 (63%)						
Location of the disease	Central portion	13 (6%)	78 (5%)	1.31	0.71–2.40	0.381			
	Others	188 (94%)	1479 (95%)						
Palpable	Yes	113 (56%)	1175 (75%)	0.42	0.31–0.56	**<0.001**			
	No	88 (44%)	382 (25%)						
MMG density	High	149 (74%)	742 (48%)	3.15	2.26–4.38	**<0.001**	2.49	1.67–3.76	**<0.001**
	Low	52 (26%)	815 (52%)						
Surgery	Total mastectomy	87 (43%)	841 (54%)	0.65	0.48–0.87	**0.004**			
	Partial resection	114 (57%)	716 (46%)						
Histology	Invasive	138 (69%)	1295 (83%)	0.45	0.32–0.62	**<0.001**	0.50	0.35–0.73	**<0.001**
	Non-invasive	63 (31%)	262 (17%)						
Histological type									
(Invasive)	NST	118 (86%)[a]	1108 (86%)[a]	1.00	0.61–1.65	0.995			
	Special type	20 (14%)[a]	187 (14%)[a]						
(Non-invasive)	Comedo	10 (16%)**	88 (34%)**	0.38	0.18–0.78	**0.008**			
	Non-comedo	53 (84%)**	174 (66%)**						
Tumor size in total[b] (mm)	>16	92 (46%)	1027 (66%)	0.44	0.32–0.59	**<0.001**	0.48	0.35–0.67	**<0.001**
	≤16	109 (54%)	530 (34%)						
Tumor size (mean, mm)									
(Invasive)	(range)	14.1 (0.4–110)	21.2 (0.2–139)	0.00	0.00–449	**<0.001**			
(Non-invasive)	(range)	25.7 (1–65)	29.4 (1–150)	0.33	0.05–2.21	0.241			
Lymph node involvement	Yes	19 (9%)	323 (21%)	0.40	0.25–0.65	**<0.001**	0.48	0.26–0.81	**0.005**
	No	175 (87%)	1194 (77%)						
	Not evaluated	7 (4%)	40 (3%)						
Tumor grade	High	13 (6%)	243 (16%)	0.37	0.21–0.66	**<0.001**	0.56	0.28–1.01	0.056
	Intermediate/low	177 (88%)	1231 (79%)						
	Not evaluated	11 (5%)	83 (5%)						
ER	Positive	187 (93%)	1316 (85%)	2.45	1.40–4.28	**0.002**	1.48	0.80–2.93	0.224
	Negative	14 (7%)	241 (15%)						
PgR	Positive	177 (88%)	1173 (75%)	2.41	1.55–3.75	**<0.001**			
	Negative	24 (12%)	384 (25%)						
HER2	Positive	29 (14%)	271 (17%)	0.80	0.53–1.21	0.292	0.86	0.53–1.37	0.531
	Negative	172 (86%)	1286 (83%)						
Subtype	Luminal HER2-negative	168 (84%)	1201 (77%)	0.38[c]	0.16–0.77	**0.006**			
	Luminal HER2-positive	19 (9%)	125 (8%)						
	HER2	7 (3%)	97 (6%)						
	Triple negative	7 (3%)	134 (9%)						
Ki67 labeling index (%)	>43	37 (18%)	428 (28%)	0.59	0.40–0.85	**0.004**			
	≤43	163 (82%)	1115 (72%)						

BMI: body mass index, NST: no special type, OR: odds ratio, CI: confidence interval.
All ORs were calculated with the MG-positive group as the baseline.
[a] Rates in invasive tumors, **rates in non-invasive tumors.
[b] Indicates largest tumor dimension, e.g. the size of the intraductal component was employed if it was larger than the invasive component.
[c] comparison between triple negative and other subtypes.

0.62, and 0.53 (Supplementary Fig. 1). For the full-model analysis, we first selected variables according to their clinical significance. Age, BMI, MG density, histology (invasive or non-invasive), pathological tumor size in total, lymph node metastasis, tumor grade, ER and HER2 status were thus chosen. All odds ratios were calculated using the MG-positive group as the baseline.

To assess the outcomes of patients with invasive disease according to MG categorization, propensity score matching was conducted. For calculating propensity scores, we selected age, breast density, extent of tumor invasion, lymph node metastasis, ER and HER2 status, and whether chemotherapies were being administered. For matching, 0.20 was employed as the caliper width, with logit transformation of the data. Kaplan-Meier curves were estimated and the log-rank test was applied for comparisons of the survival distributions of the two patient groups. A $p < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. MG categorization

There were 201 cases (11.4%) who had no cancer-specific findings on MG (MG-negative group) among all subjects. The remaining 1557 cases (88.6%) had findings possibly indicative of cancer such as mass, calcification, focal asymmetric density and distortion, and were thus categorized as the MG-positive group. These findings are presented in detail in Supplementary Table 1.

Table 3
Clinicopathological features according to MG categorization: Invasive carcinoma (n = 1433).

Variables		MG-negative group	MG-positive group	Univariate			Multivariate		
				OR	95% CI	p value	OR	95% CI	p value
n		138	1295						
Age	>53	36 (26%)	710 (55%)	0.29	0.19–0.43	**<0.001**	0.43	0.27–0.68	**<0.001**
	≤53	102 (74%)	585 (45%)						
Menopause	Yes	39 (29%)	719 (56%)	0.3	0.20–0.44	**<0.001**			
	No	97 (70%)	544 (42%)						
	Unknown	2 (1%)	32 [2]						
BMI	>23	32 (23%)	477 (37%)	0.52	0.34–0.77	**0.001**	0.79	0.49–1.22	0.292
	≤23	106 (77%)	818 (63%)						
Location of the disease	Central portion	7 (5%)	66 (5%)	0.99	0.41–2.07	0.989			
	Others	131 (95%)	1229 (95%)						
Palpable	Yes	91 (66%)	1064 (82%)	0.42	0.29–0.62	**<0.001**			
	No	47 (34%)	231 (18%)						
MMG density	High	108 (78%)	613 (47%)	4.01	2.67–6.20	**<0.001**	2.6	1.61–4.30	**<0.001**
	Low	30 (22%)	682 (53%)						
Surgery	Total mastectomy	56 (41%)	701 (54%)	0.58	0.40–0.83	**0.003**			
	Partial resection	82 (59%)	594 (46%)						
Histological type	NST	118 (86%)	1106 (86%)	1	0.59–1.61	0.995			
	Special type	20 (14%)	187 (14%)						
Tumor size (mm)	>16	56 (41%)	869 (67%)	0.33	0.23–0.48	**<0.001**	0.39	0.25–0.59	**<0.001**
	≤16	82 (59%)	426 (33%)						
Lymph node involvement	Yes	17 (12%)	321 (25%)	0.43	0.25–0.71	**<0.001**	0.49	0.26–0.85	**0.011**
	No	117 (85%)	958 (74%)						
	Not evaluated	3 (2%)	16 (1%)						
Tumor grade	High	8 (6%)	209 (16%)	0.32	0.14–0.61	**<0.001**	0.43	0.18–0.91	**0.027**
	Intermediate/low	123 (89%)	1014 (78%)						
	Not evaluated	7 (5%)	72 (6%)						
ER	Positive	129 (93%)	1105 (85%)	2.47	1.30–5.30	**0.004**	1.16	0.56–2.69	0.702
	Negative	9 (7%)	190 (15%)						
PgR	Positive	126 (91%)	980 (76%)	3.38	1.92–6.52	**<0.001**			
	Negative	12 (9%)	315 (24%)						
HER2	Positive	15 (11%)	194 (15%)	0.7	0.38–1.18	0.184	0.82	0.43–1.49	0.536
	Negative	123 (89%)	1101 (85%)						
Subtype	Luminal HER2-negative	117 (85%)	1023 (79%)	0.46[a]	0.18–0.97	**0.042**			
	Luminal HER2-positive	12 (9%)	90 (7%)						
	HER2	3 (2%)	65 (5%)						
	Triple negative	6 (4%)	117 (9%)						
Ki67 labeling index (%)	>38	37 (27%)	487 (38%)	0.6	0.40–0.88	**0.009**			
	≤38	101 (73%)	798 (62%)						
	Not evaluated	0 (0%)	10 (1%)						

BMI: body mass index, NST: no special type, OR: odds ratio, CI: confidence interval.
All ORs were calculated with the MG-positive group as the baseline.
[a] Comparison between triple negative and other subtypes.

3.2. Clinicopathological features of the MG-negative cases including breast density

Median ages of the MG-negative and the MG-positive groups were 48.0 (range: 30–84) and 55.0 (24–93) years, respectively, and the MG-negative group was significantly younger ($p < 0.001$). Comparisons of clinicopathological features between these two groups are shown in Table 2. As to breast density, 74% (149 cases) of the MG-negative group had dense breast tissues and this rate was significantly higher than the 48% (742 cases) in the MG-positive group ($p < 0.001$). Univariate analysis also revealed that there were statistically significant differences in age, menopausal status, BMI, detection of breast cancer by palpation, surgical procedure, histology, tumor size, lymph node involvement, tumor grade, ER, PgR, intrinsic subtype, and the Ki67 labeling index. There was, however, no difference in factors such as tumor location and HER2 status between these two patient groups. Multivariate analysis revealed that age, MG density, histology (invasive versus non-invasive), total tumor size and lymph node involvement were independent factors differing between the MG-negative and MG-positive groups. More patients in the MG-negative group were young ($p = 0.007$) and showed high breast density on MG ($p < 0.001$). MG-negative patients more commonly had non-invasive disease ($p < 0.001$), smaller tumors ($p < 0.001$), and less lymph node metastasis ($p = 0.005$).

Next, for further analysis, the results indicated above were categorized into invasive and non-invasive tumors (Tables 3 and 4). In patients with invasive disease, multivariate analysis revealed statistically significant differences in age, MG density, tumor size, lymph node involvement and tumor grade between the MG-negative and MG-positive groups ($p < 0.001$, <0.001, <0.001, 0.011 and 0.027, respectively). There were, however, no differences in either ER or HER2 status between the two groups. As to patients with non-invasive disease, only MG density and comedo type were independent factors differing between the MG-negative and MG-positive groups ($p = 0.042$ and 0.013, respectively). Age and tumor size showed no associations on multivariate analysis.

3.3. Motivations for visiting the hospital according to MG categorization

Next, we compared motivations for visiting the hospital according to MG categorization. Fig. 1A indicates that 60% of MG-negative tumors were detected by screening US. The rate was higher than that in the MG-positive group (Fig. 1B), although the latter includes cases detected by US and/or MG. Next, we compared

Table 4
Clinicopathological features according to MG categorization: Non-invasive carcinoma (n = 325).

Variables		MG-negative group	MG-positive group	Univariate			Multivariate		
				OR	95% CI	p value	OR	95% CI	p value
n		63	262						
Age	>54	27 (43%)	114 (44%)	0.97	0.56–1.70	0.925	1.61	0.79–3.29	0.188
	≤54	36 (57%)	148 (56%)						
Menopause	Yes	32 (51%)	130 (50%)	1.1	0.63–1.93	0.730			
	No	29 (46%)	130 (50%)						
	Unknown	2 (3%)	2 (1%)						
BMI	>23	19 (30%)	101 (39%)	0.69	0.38–1.25	0.217	0.89	0.46–1.73	0.730
	≤23	44 (70%)	161 (61%)						
Location of the disease	Central portion	6 (10%)	12 (5%)	2.19	0.79–6.09	0.132			
	Others	57 (90%)	250 (95%)						
Palpable	Yes	22 (35%)	111 (42%)	0.73	0.41–1.29	0.282			
	No	41 (65%)	151 (58%)						
MMG density	High	41 (65%)	129 (49%)	1.92	1.08–3.40	**0.025**	2.19	1.03–4.65	**0.042**
	Low	22 (35%)	133 (51%)						
Surgery	Total mastectomy	31 (49%)	140 (53%)	0.84	0.48–1.46	0.546			
	Partial resection	32 (51%)	122 (47%)						
Histological type	Comedo	10 (16%)	88 (34%)	0.37	0.18–0.77	**0.008**	0.37	0.17–0.81	**0.013**
	Non-comedo	53 (84%)	174 (66%)						
Tumor size (mm)	>28	27 (43%)	110 (42%)	1.04	0.59–1.81	0.900	1.19	0.65–2.21	0.574
	≤28	36 (57%)	152 (58%)						
Lymph node involvement	Yes	2 (3%)	2 (1%)	4.07	0.56–29.5	0.165	5.25	0.62–44.3	0.128
	No	58 (92%)	236 (90%)						
	Not evaluated	3 (5%)	24 (9%)						
Tumor grade	High	5 (8%)	34 (13%)	0.59	0.22–1.58	0.295			
	Intermediate/low	54 (86%)	217 (83%)						
	Not evaluated	4 (6%)	11 (4%)						
ER	Positive	58 (92%)	211 (81%)	2.8	1.07–7.35	**0.036**	2.84	0.94–8.56	0.063
	Negative	5 (8%)	51 (19%)						
PgR	Positive	51 (81%)	193 (74%)	1.52	0.76–3.02	0.232			
	Negative	12 (19%)	69 (26%)						
HER2	Positive	14 (22%)	77 (29%)	0.69	0.36–1.32	0.257	0.97	0.45–2.05	0.930
	Negative	49 (78%)	185 (71%)						
Subtype	Luminal HER2-negative	51 (81%)	178 (68%)	0.23[a]	0.03–1.78	0.160			
	Luminal HER2-positive	7 (11%)	35 (13%)						
	HER2	4 (6%)	32 (12%)						
	Triple negative	1 (2%)	17 (6%)						
Ki67 labeling index (%)	>26	26 (41%)	100 (38%)	1.14	0.65–2.00	0.646			
	≤26	36 (57%)	158 (60%)						
	Not evaluated	1 (2%)	4 (2%)						

BMI: body mass index, OR: odds ratio, CI: confidence interval.
All ORs were calculated with the MG-positive group as the baseline.
[a] Comparison between triple negative and other subtypes.

Table 5
Sonographic features according to MG categorization.

		MG-negative group (n = 200)		MG-positive group (n = 200)		p value[a]
Mass lesion (n, %)		167 (84%)		163 (82%)		
	Solid mass		155 (78%)		160 (80%)	
	Intracystic mass		11 (6%)		3 (2%)	0.061
	Cyst alone		1 (0.5%)		0 (0%)	
Non-mass lesion (n, %)		33 (16%)		31 (16%)		
	Ductal ectasia		11 (6%)		1 (0.5%)	
	Low echoic lesion		20 (10%)		27 (14%)	0.009
	Architectural distortion		2 (1%)		3 (2%)	
No findings (n, %)		0 (0%)		6 (3%)		

[a] Comparisons between MG-negative and positive groups by the Chi-squared test.

clinicopathological factors according to the motivations for visiting the hospital, i.e. screening-detected vs subjective symptoms (Supplementary Table 2). In the MG-negative group, only tumor size differed between these two groups, as the mean size of screening US-detected tumors was smaller than that of the tumors of patients with subjective symptoms (p = 0.009). These tumors were smaller than in the MG-positive group. Meanwhile, there were no differences in tumor characteristics such as tumor grade and the Ki67 labeling index between screening-detected and subjective symptoms in the MG-negative group. On the contrary, in the MG-positive group, tumors of patients with subjective symptoms were basically more advanced and highly malignant.

3.4. US findings of MG-negative group

We also examined US findings of the MG-negative cases. Of the 201 cases in the MG-negative group, we were unable to access the US imaging records for one patient. All 200 cases, whose records

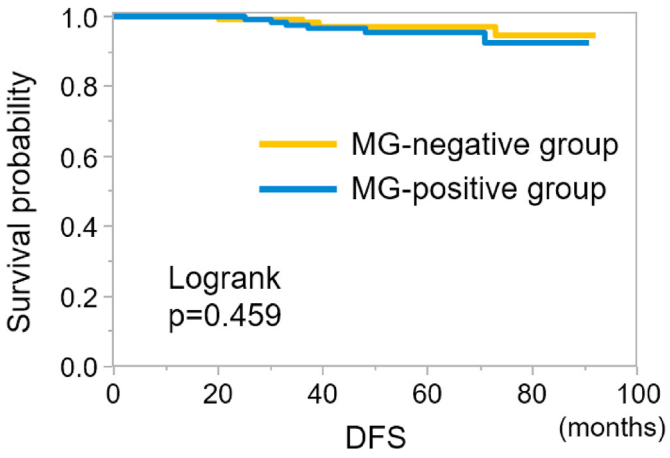

Fig. 2. Kaplan-Meier curves of disease-free survival according to MG categorization (n = 266)
Kaplan-Meier curves for disease-free survival (DFS) in 133 paired patients with invasive disease according to MG categorization are shown. The MG-negative and MG-positive groups are indicated in yellow and blue, respectively.

were available, had US findings and the details were as follows; solid mass in 155 (78%), intracystic mass in 11 (6%), cystic lesion only in one (0.5%), ductal ectasia in 11 (6%), low echoic area in 20 (10%), and architectural distortion in 2 (1%) cases (Table 5). For comparison, we randomly chose 200 cases from the MG-positive group. There was no difference in the rate of mass versus non-mass lesions between the two groups. However, in non-mass lesions, distributions of US findings differed between these two groups ($p = 0.009$), as ductal ectasia was more common in the MG-negative group.

3.5. Patient outcomes according to MG categorization

Finally, we examined the outcomes of patients with invasive disease according to the MG categorization. First, patient outcomes of 137 cases with invasive disease from the MG-negative group and 274 cases from the MG-positive group, randomly selected for comparison, were investigated. As mentioned in the Methods section, propensity score matching was then conducted based on age, breast density, the extent of tumor invasion, lymph node metastasis, ER and HER2 statuses, and whether adjuvant chemotherapies were being administered. Two hundred and sixty-six patients (133 pairs) were matched and the clinicopathological features of these patients are shown in Supplementary Table 3. Of these 266 patients, 10 developed distant metastasis during the 54-month mean follow-up period (range: 10–92 months). Fig. 2 shows Kaplan-Meier curves of disease-free survival according to MG categorization. There was no statistically significant difference between the MG-negative and MG-positive groups ($p = 0.459$).

4. Discussion

Patients in the MG-negative group had higher breast density on MG, smaller tumors and less lymph node involvement than those in the MG-positive group. Moreover, low tumor grade was significantly more often observed in the MG-negative group patients. Our data are apparently different from the findings of previous reports showing more advanced diseases with larger tumors and more lymph node involvement in patients with dense breast tissues [12,22,23]. However, this is attributable to differences among study designs. In previous studies focusing on MG densities, breast cancer in dense breasts might have initially been masked but was later found. In our study, we focused on whether or not the lesions detected on MG might raise suspicion of malignancies apart from the breast density status. MG-negative patients frequently had dense breast tissues, probably a major factor underlying masking of the lesion, but their cancers were in early stages due to detection by US or recognition by the patients themselves when symptomatic.

In the current study, we found that many of the MG-negative tumors had been detected by screening US. US had a major role in detecting tumors in patients free of symptoms in this group, probably at earlier stages, i.e. before the tumors became palpable. Although not all patients in this population had dense breast tissues, our data indicate the usefulness of US screening, as suggested by the aforementioned large randomized study [11]. Although it has become clear that additional US improves the detection rate of breast cancer, this is not currently recommended by the government in Japan. This is due mainly to the improvement in the survival rate having not yet been clarified. Therefore, how to combine US with MG-based screening, not only in Japan but worldwide, is now a critical issue. A recent meta-analysis revealed a slight decrease in cancer specificity with the addition of US to MG for examining in women with dense breasts [24]. Meanwhile, tumor characteristics such as tumor grade and the Ki67 labeling index were lower in the MG-negative than in the MG-positive group, regardless of the reasons given for visiting the hospital. These data raise the possibility that US may not have to be coupled with every MG screening because tumors with such low malignancy might grow slowly. This may correspond to the possibly low significance of finding so-called “low grade” ductal carcinoma in situ on MG screening [25,26]. As for the frequency of US, once every two/three times that MG is performed also having the subjects undergo US might be both practical and efficient. We hope that our data will be useful in such discussions of the roles of US in screening programs, including efficacy and feasibility.

As to US findings, there was no difference in mass versus non-mass lesions between the MG-negative and MG-positive groups. Intracystic mass and ductal ectasia were more frequent in the MG-negative group, probably reflecting higher rates of non-invasive disease in this population. We examined the characteristics of tumors, detectable by US but not on MG.

The limitations of this study include the design and relatively small number of cases. Our cohort included patients with subjective symptoms and those detected by screening. Thus, more samples limited to screening-detected patients should be employed for further investigation. As to US-screening detection, data obtained from women who were referred to our hospital based on US screening but found to have benign diseases should also be examined, to evaluate the efficacy of this approach. To assess the usefulness of additional US for screening programs, a large dataset at the national level will need to be validated, including information such as frequency, cancer detection rates, and contributions to improvement of patient outcomes. There was no difference in patient outcomes between the MG-negative and MG-positive groups. However, our sample numbers markedly diminished after propensity score matching based on certain clinically important factors such as age and differences in systemic treatments. Thus, further studies with a larger number of patients are needed before conclusions can be drawn regarding the utility of adding US to MG screening.

In summary, patients in the MG-negative group frequently had dense breasts and the majority of tumors in this group were found by US screening. Moreover, their breast cancers were detected at a relatively early stage and showed relatively low malignancy, regardless of motives of visiting the hospital. Further investigations are warranted to confirm the usefulness of adding US to MG screening.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.breast.2020.11.010.

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