Diagnostic Performance Improvement with Combined use of Proteomics Biomarker Assay and Breast Ultrasound

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Abstract

Purpose: To investigate the combined use of blood-based 3-protein signature and breast ultrasound (US) for validating US detected lesions.

Methods: From July 2011 to April 2020, women who underwent whole-breast US within at least 6 months from sampling period were retrospectively included. Blood-based 3-protein signature (Mastocheck®) value and US findings were evaluated. Following outcome measures were compared between US alone and the combination of Mastocheck® value with US: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), area under the receiver operating characteristic curve (AUC), and biopsy rate.

Results: Among the 237 women included, 59 (24.9%) were healthy individuals and 178 (75.1%) cancer patients. Mean size of cancers was 1.2±0.8 cm. Median value of Mastocheck® was significantly different between non-malignant (-0.24, interquartile range [IQR], -0.48, -0.03) and malignant lesions (0.55, IQR, -0.03, 1.42) ($P < .001$). Utilizing Mastocheck® value with US increased the AUC from 0.67 (95% confidence interval [CI], 0.61, 0.73) to 0.81 (95% CI: 0.75, 0.88; $P < .001$), specificity from 35.6% (95% CI: 23.4, 47.8) to 64.4% (95% CI: 52.2, 76.6; $P < .001$) without loss in sensitivity. PPV was increased from 82.2% (95% CI: 77.1, 87.3) to 89.3% (95% CI: 85.0, 93.6; $P < .001$), and biopsy rate was significantly decreased from 79.3% (188/237) to 72.1% (171/237) ($P < .001$). Consistent improvements in specificity, PPV, and AUC were observed in asymptomatic women and in those with normal/benign mammographic findings.

Conclusion: Mastocheck® is an effective tool that can be used with US to improve diagnostic specificity and reduce false-positive findings and unnecessary biopsies.

Introduction

Annual mammography for breast cancer screening has led to a substantial reduction in morbidity and mortality, with 41.0% mortality reduction and 25.0% drop in the incidence of advanced breast cancers [1, 2]. However, mammography is limited by low sensitivity, especially in women with dense breasts [3]. Multiple trials have shown that supplemental breast ultrasound (US) significantly increases the detection of node-negative invasive breast cancer in women with mammographically dense breasts, increasing cancer detection by 3.5/1,000 in single-center studies [4–6] and 4.4/1,000 in multi-center trials [7–9]. In addition, breast US is widely used as a diagnostic tool for evaluating mammographically detected masses [10], palpable lesions [11] and guiding biopsies [12]. It is relatively inexpensive and well-tolerated by the patient because there is no risk of ionizing radiation exposure or need for intravenous contrast material injection [13]. However, it has been criticized for its relatively low specificity, leading to many recalls and biopsies of benign lesions. As a result, there has been much interest in developments primarily aimed at increasing the specificity of US [14–18].

Liquid biopsy, an innovative technology that is a noninvasive method involving isolation of peripheral blood and analysis of cancer related circulating tumor biomarkers [19–25]. These tumor biomarkers serve for screening, diagnosis, treatment planning, and surveillance of many tumor types [21]. Over the last few decades, many researchers have investigated whether non-invasive body fluid-based tests can be used for early detection of breast cancer, and thus to overcome the limitations of mammography screening [26]. However, the data on using liquid biopsy as a screening tool in breast cancer are limited [27–30]. According to a recent publication, a blood-based 3-protein signature derived from multiple reaction monitoring (MRM)-based proteomic assay showed sensitivity, specificity, and accuracy of 71.6 %, 85.3%, and 77.0%, respectively, with an area under the receiver operating characteristic curve (AUC) of 0.83, which is specific for breast cancers and no other types of malignancies including thyroid, pancreatic, lung, and colon cancers [31]. In addition, the results of a blood-based 3-protein signature alone were superior compared to that of only mammography, as interpreted by non-dedicated general radiologists; furthermore, the diagnostic performance was improved by using a combination of mammography and a blood-based 3-protein signature [32]. Although this method showed a potential to be used as a test for breast cancer screening, as well as a complementary tool for preexisting diagnostic imaging modalities, to the best of our knowledge, there have been no studies comparing the value of a proteomics biomarker and breast US for the evaluation of breast masses or of its additional diagnostic value when used in combination with breast US. If a blood-based proteomics biomarker is used as an adjunct to breast US to improve the specificity and overall accuracy, it could reduce false-positive recall and improve patient management. Therefore, the purpose of this study is to determine whether adding a non-invasive blood-based proteomics biomarker assay could improve the accuracy of breast US by reducing false-positive findings, without loss of sensitivity for detection of breast cancer.

Methods

The institutional review board approved this retrospective study and waived the requirement for obtaining informed consent from the patients (IRB No. 2109-098-1255).

Study Population

From July 2011 to April 2020, we performed a retrospective search for women who had blood samples collected to evaluate blood-based 3-protein signature with institutional review board approval (IRB No. H-1807-057-957) and underwent bilateral whole-breast US within at least 6 months from

the sampling period. The subgroup of this study population has been reported in previous studies [31-33]. Patients were excluded for the following reasons: undergone breast surgery within the previous 12 months, personal history of breast cancer, and no follow-up for at least 12 months to confirm the stability of the lesion.

Imaging Acquisition and Interpretation

All imaging data were obtained prospectively as part of our routine clinical practice and stored in the Picture Archiving and Communication System (PACS). Whole-breast US examinations were performed by one of 15 dedicated breast radiologists, with 1-30 years' experience with breast US. Whole-breast US was performed using a 14-6 MHz linear transducer (EUB-8500 Hitachi Medical, Chiba, Japan) and 15-4 MHz linear transducer (Aixplorer; Supersonic Image, Aix-en-Provence, France). For a standard US examination, representative images at 12, 3, 6, and 9 o'clock positions, as well as retro areolar positions, were documented. When a lesion other than a simple cyst was found, its clock face location and distance from the nipple were documented. Images in two orthogonal planes, including radial, anti-radial, transverse, and longitudinal planes, and their maximal diameter measurements were recorded. Color Doppler or elastographic images were obtained when deemed necessary by the radiologist. The final assessment categories were assigned by the radiologists performing US examination, and the assessment was recorded on the basis of the expanded seven Breast Imaging-Reporting and Data System (BI-RADS) categories: category 1, negative; category 2, benign; category 3, probably benign; category 4A, low suspicion; category 4B, moderate suspicion; category 4C, high suspicion; and category 5, highly suggestive of malignancy [34]. Cases assigned a BI-RADS category 3 or higher were considered positive, and BI-RADS category 1 or 2 were considered a negative result.

Preparation of Blood Samples

Mastocheck® (Bertis, Geonggi-do, Korea) is a blood-based biomarker that screens for the development of breast cancer through algorithmic calculations of three plasma protein levels specific to breast cancer: carbonic anhydrase 1 (CAH1), neural cell adhesion molecule L1-like protein (NCHL1), and apolipoprotein C-1 [APOC1]. [33, 35]. This biomarker has been approved for use in humans by the Korean Ministry of Food and Drug Safety in January 2019. The quantitative estimation of the Mastocheck® value is described in the Appendix. A previous study reported 0.0668 as an optimal cut-off value of Mastocheck® for breast cancer diagnosis, with a sensitivity and specificity of 67.0% and 82.0%, respectively. Further validation studies showed sensitivity and specificity of 71.6% and 85.3%, respectively, with the cut-off value mentioned above (31, 32). Based on this, a positive result was defined as a Mastocheck® value of $\geq$ 0.0668 and a negative result of < 0.0668 [32].

Combined assessment of US and Mastocheck®

Two breast radiologists (J. M. C. and S. M. H. with 14 and 8 years' experience in breast imaging, respectively) retrospectively reviewed breast US examinations, and the US BI-RADS category was re-categorized after consideration of Mastocheck® values, blinded to clinical and pathological information. Since most diagnostic dilemmas occur for probably benign (BI-RADS category 3) or low suspicion findings (BI-RADS category 4A), we applied Mastocheck® values to BI-RADS category re-assessments for lesions near the threshold level of biopsy (i.e., BI-RADS category 3 or 4A lesions). For combined assessment of US and Mastocheck®, the BI-RADS category on US was downgraded by one category if the patient had a Mastocheck® value below 0.0668 to determine whether there is an increase in the specificity of breast US and to decrease unnecessary benign biopsy rate [32]. BI-RADS category 4A lesions were downgraded to BI-RADS category 3. If Mastocheck® was negative for BI-RADS category 3 lesions, it was downgraded by one category (BI-RADS category 2). For lesions with BI-RADS category 4B or higher, Mastocheck® values were not considered for combined assessment, and the BI-RADS final assessment was not changed.

Reference Standards

The reference standard was based on histopathologic results of biopsy (n=3), surgical result (n=178), or stability at follow-up imaging for more than 12 months (n=56). Biopsy results showing breast cancer (i.e., invasive carcinoma or ductal carcinoma in situ) were considered malignant. Lesions that remained stable or decreased at 12 months follow-up US without biopsy were considered nonmalignant.

Statistical Analysis

We calculated the sensitivity, specificity, PPV, negative predictive value (NPV), and AUC of Mastocheck® alone, US alone, and combined assessment using the method of DeLong et al [36]. The increase in AUC was compared between subgroups using an independent *t*-test on the basis of estimates and standard errors for AUC increments. Sensitivity and specificity were compared using McNemar's test. A generalized estimating equation was used to compare the PPV and NPV. The biopsy rate, defined as the number of breast lesions above BI-RADS category 4A (recommended for biopsy) divided by the total number of breast lesions, was also compared. All tests were two-sided, and *P* values less than .05 were considered to indicate

statistical significance, and we also reported 95% confidence intervals (CI). Statistical analyses were performed using the statistical software SAS version 9.4 (SAS Institute, Cary, NC USA).

Results

Study Population Characteristics

Among 260 women who had undergone blood sampling for evaluation of Mastocheck® value and whole-breast US, we excluded 23 because of a personal history of breast cancer (n=1), recent surgical excision (n=5), and no follow-up for at least 12 months (n=17). Finally, 237 women (median age, 51 years; interquartile age range [IQR], 45-58 years) were included, and 59 (24.9%) healthy individuals (median age, 54 years; IQR, 47-60 years) and 178 (75.1%) breast cancer patients (median age, 51 years; IQR, 44-58 years) (Fig. 1). A total of 138 (58.2%, 138/237) women were asymptomatic, and 99 (41.8%, 99/237) had breast-related symptoms. A total of 192 (81.0%, 192/237) women had dense breasts, 41 (17.3%, 41/237) had fatty breasts on mammography, and 4 (1.7%, 4/237) had no available mammography. The characteristics of the study population are summarized in Table 1. Among 178 patients with breast cancer diagnosis, 142 (79.8%) had invasive ductal cancer, 11 (6.2%) invasive lobular cancer, 17 (9.5%) ductal carcinoma in situ, 3 (1.7%) mixed invasive ductal and lobular cancer, 2 (1.1%) mucinous carcinoma, 2 (1.1%) metaplastic carcinoma, and 1 (0.6%) intraductal papillary carcinoma. The mean size of 178 cancers was 1.2±0.8 cm (median, 1.5 cm; range, 0-4.0 cm). Most were stage I (50.6%, 90/178) and stage II (39.3%, 70/178). Benign lesions were fibroadenoma (n=2), intraductal papilloma (n=1); and follow-up imaging was performed for 56 lesions and were stable with a follow-up period of 13.5 months (range, 12.1-25.2 months).

Table 1
Baseline characteristics of the study patients

Parameter	Total (n=237)	Nonmalignant (n=59)	Malignant (n=178)
Age (years)			
Median (Interquartile range)	51 (45-58)	54 (47-60)	51 (44-58)
Mammographic breast density			
BI-RADS A or B, fatty	41 (17.3)	13 (22.0)	28 (15.7)
BI-RADS C or D, dense	192 (81.0)	42 (71.2)	150 (84.3)
Not available	4 (1.7)	4 (6.8)	0 (0.0)
Symptom			
No	138 (58.2)	54 (91.5)	84 (47.2)
Yes	99 (41.8)	5 (8.5)	94 (52.8)
American Joint Committee on Cancer Stage			
0			17 (9.5)
I			90 (50.6)
II			70 (39.3)
III			1 (0.6)
Histologic type			
Ductal carcinoma in situ			17 (9.5)
Invasive ductal			142 (79.8)
Invasive lobular			11 (6.2)
Invasive ductal and lobular			3 (1.7)
Others			5 (2.8)
Note. —Data in parentheses indicate the percentages			
BI-RADS = Breast Imaging Reporting and Data System			

Assessment On Breast Us And Mastocheck®

The BI-RADS final assessment category and Mastocheck® values are listed in Table 2. On US, 98.9% (176/178) of malignant masses were assessed as test-positive, and 64.4% (38/59) of non-malignant lesions as test-positive. There were two (1.1%, 2/178) BI-RADS category 2 and two (1.1%, 2/178) BI-RADS category 3 breast cancers manifesting suspicious calcifications on mammography that were not visible on US. The results of Mastocheck® showed positive results with values higher than 0.0668 in 126 lesions (70.8%, 126/178) among breast cancer patients, whereas it showed negative results with values lower than 0.0668 in 46 lesions (78.0%, 46/59) in nonmalignant cases ($P < .001$). For nonmalignant lesions, the median value was -0.24 (IQR, -0.48, -0.03) and for malignant lesions, the median value was 0.55 (IQR, -0.03, 1.42) with significant difference ($P < .001$).

Table 2
Assessment on Mastocheck®, US, and Combined Mastocheck® with US

Parameter	Total (n=237)	Nonmalignant (n=59)	Malignant (n=178)	*P* value
US Assessment				<.001
Negative (BI-RADS Category 1, 2)	23 (9.7)	21 (35.6)	2 (1.1)	
Positive (BI-RADS Category ≥3)	214 (90.3)	38 (64.4)	176 (98.9)	
3	26 (11.0)	24 (40.7)	2 (1.1)	
4A	35 (14.8)	14 (23.7)	21 (11.8)	
4B	25 (10.5)	0 (0)	25 (14.0)	
4C	34 (14.3)	0 (0)	34 (19.1)	
5	94 (39.7)	0 (0)	94 (52.8)	
Mastocheck® Value				<.001
Median (Interquartile range)	0.26 (-0.25, 1.13)	-0.24 (-0.48, -0.03)	0.55 (-0.03, 1.42)	
Negative (value < 0.0668)	98 (41.4)	46 (78.0)	52 (29.2)	
Positive (value ≥ 0.0668)	139 (58.6)	13 (22.0)	126 (70.8)	
Combined Assessment*				<.001
Negative	40 (16.9)	38 (64.4)	2 (1.1)	
Positive	197 (83.1)	21 (35.6)	176 (98.9)	
Note. —Data in parentheses indicate the percentages				
BI-RADS = Breast Imaging Reporting and Data System				
*For combined assessment, BI-RADS category 3 or 4A masses are downgraded to BI-RADS category 2 or 3 if Mastocheck® value is less than 0.0668.				

Performance Of Mastocheck®, Us, And Combined Mastocheck® With Us

For breast US, with BI-RADS category of 3 or higher considered to be positive, the AUC of breast US alone was 0.67 (95%CI, 0.61, 0.73). Sensitivity, specificity, PPV, and NPV were 98.9% (95% CI: 97.3, 100.0), 35.6% (95% CI: 23.4, 47.8), 82.2% (95% CI: 77.1, 87.3), and 91.3% (95% CI: 79.8, 100.0), respectively. Mastocheck® showed AUC of 0.74 (95% CI, 0.68, 0.81), sensitivity of 70.8% (95% CI: 64.1, 77.5), specificity of 77.9% (95% CI, 67.4, 88.5), PPV of 90.6% (95% CI, 85.8, 95.5), and NPV of 46.9% (95% CI, 37.0, 56.8) (Table 3). By addition of Mastocheck® value to breast US assessment, the AUC was increased from 0.67 (95% CI, 0.61, 0.73) to 0.81 (95% CI 0.75, 0.88; $P < .001$), specificity was increased from 35.6% (95% CI: 23.4, 47.8) to 64.4% (95% CI: 52.2, 76.6; $P < .001$) without a loss in sensitivity (Fig. 2); PPV was also increased from 82.2% (95% CI: 77.1, 87.3) to 89.3% (95% CI: 85.0, 93.6; $P < .001$) (Fig. 3). The biopsy rate was significantly decreased from 79.3% (188/237) to 72.1% (171/237) ($P < .001$). By downgrading one category according to the Mastocheck® result showing lower value than 0.0668, the number of positive cases was decreased from 38 to 21 lesions (44.8% decrease) and 85.7% (12/14) of unnecessary biopsies for non-malignant lesions could be avoided (Fig. 4). Five cancers that were initially classified as BI-RADS category 4A on breast US were downgraded to BI-RADS category 3 with negative values on Mastocheck®. They were two 0.5-cm and 0.2-cm invasive ductal carcinoma, two 0.5-cm invasive lobular carcinoma, and one ductal carcinoma in situ (Table 4, Fig. 5).

Table 3
Diagnostic Performance of Mastocheck®, US, and Combined Mastocheck® with US

Parameter	Mastocheck®	US	Combined*	*P* value for Mastocheck® vs. US	*P* value for Mastocheck® vs. Combined	*P* value for US vs. Combined
AUC	0.74 (0.68, 0.81)	0.67 (0.61, 0.73)	0.81 (0.75, 0.88)	.104	.051	<.001
Sensitivity (%)	70.8 (64.1, 77.5) [126/178]	98.9 (97.3, 100) [176/178]	98.9 (97.3, 100) [176/178]	<.001	<.001	-
Specificity (%)	77.9 (67.4, 88.5) [46/59]	35.6 (23.4, 47.8) [21/59]	64.4 (52.2, 76.6) [38/59]	<.001	.042	<.001
PPV (%)	90.6 (85.8, 95.5) [126/139]	82.2 (77.1, 87.3) [176/214]	89.3 (85.0, 93.6) [176/197]	<.001	.692	<.001
NPV (%)	46.9 (37.0, 56.8) [46/98]	91.3 (79.8, 100.0) [21/23]	95.0 (88.2, 100) [38/40]	<.001	<.001	.491
Biopsy rate (%)		79.3 [188/237]	72.1 [171/237]	-	-	<.001

Note- Data in parentheses are 95% confidence intervals, and data in brackets are numerators and denominators.

AUC = area under the curve; PPV = positive predictive value; NPV = negative predictive value.

*For combined assessment, BI-RADS category 3 or 4A masses are downgraded to BI-RADS category 2 or 3 if Mastocheck® value is less than 0.0668.

Table 4
Characteristics of Five Breast Cancers Downgraded by combined interpretation of breast US and Mastocheck® value

Patient	Age (years)	Symptom	MG density	MG BI-RADS	US BI-RADS	Mastocheck® value	Stage of cancer	Size (cm)	Histologic Type	Grade	Hormone receptor status
1	63	No	D	2	4A	-0.3800	I	0.5	Invasive ductal	Low	ER+/PR+/HER2-
2	51	No	B	1	4A	0.0564	I	0.5	Invasive lobular	Intermediate	ER+/PR+/HER2-
3	52	No	D	1	4A	-0.5943	I	0.5	Invasive lobular	Intermediate	ER+/PR+/HER2-
4	56	Palpable	D	3	4A	-0.7231	I	0.2	Invasive ductal	Intermediate	ER+/PR+/HER2-
5	53	No	D	4	4A	-1.1326	0	0.2	DCIS	Intermediate	ER+/PR+/HER2-

BI-RADS = Breast Imaging Reporting and Data System; DCIS = ductal carcinoma in situ; ER = estrogen receptor, HER2 = human epidermal growth factor receptor 2; MG = mammography; PR = progesterone receptor; US = ultrasound.

Subgroup Analysis

To investigate whether the combined assessment showed similar performance in mimic screening setting, we performed subgroup analysis according to the presence of symptom and mammographic finding. In subgroup of 63 women with normal or benign mammographic findings, AUC of US was increased from 0.70 (95% CI: 0.62, 0.77) to 0.84 (95% CI: 0.77, 0.91; $P < .001$), specificity and PPV were also increased from 39.1 % (95% CI: 25.0, 53.2) to 67.4% (95% CI: 53.8, 80.9; $P < .001$) and from 37.8% (95% CI: 23.6, 51.9) to 53.1% (95% CI: 35.8, 70.4, $P < .001$), respectively, without loss of sensitivity with addition of Mastocheck® result. In 135 asymptomatic women, with the addition of Mastocheck® result, the AUC of US was increased from 0.66 (95% CI: 0.60, 0.73) to 0.81 (95% CI: 0.74, 0.88; $P < .001$), specificity and PPV were also increased from 35.3 % (95% CI: 22.2, 48.4) to 64.7% (95% CI: 51.6, 77.8; $P < .001$) and from 71.3% (95% CI: 63.0, 79.6) to 82.0% (95% CI: 74.5, 89.5, $P < .001$), respectively, without loss of sensitivity. The improvement in AUC, specificity, and PPV was consistently seen, 0.70 (95% CI: 0.63, 0.78) to 0.85 (95% CI: 0.77, 0.92), 40.5% (95% CI:

25.6, 55.3) to 69.1% (95% CI: 55.1, 83.0), and 37.5% (95% CI: 22.5, 52.5) to 53.6% (95% CI: 35.1, 72.0), respectively, in 57 asymptomatic women with normal or benign findings on mammographic examinations (all, *P* < .001) (Table 5, Supplementary Fig.).

Table 5
Diagnostic Performance of Mastocheck®, US, and Combined Mastocheck® with US in Subgroups According to Mammographic Finding and Associated Symptom

Parameter	Mastocheck®	US	Combined*	*P* value for Mastocheck® vs. US	*P* value for Mastocheck® vs. Combined	*P* value for US vs. Combined
Mammography normal/benign (n=63)						
AUC	0.67 (0.54, 0.81)	0.70 (0.62, 0.77)	0.84 (0.77, 0.91)	0.782	0.020	<.001
Sensitivity (%)	58.8 (35.4, 82.2) [10/17]	100.0 (100.0) [17/17]	100.0 (100.0) [17/17]	-	-	-
Specificity (%)	76.1 (63.7, 88.4) [35/46]	39.1 (25.0, 53.2) [18/46]	67.4 (53.8, 80.9) [31/46]	<.001	0.201	<.001
PPV (%)	47.6 (26.3, 68.9) [10/21]	37.8 (23.6, 51.9) [17/45]	53.1 (35.8, 70.4) [17/32]	0.490	0.381	<.001
NPV (%)	83.3 (72.0, 94.6) [35/42]	100.0 (100.0) [18/18]	100.0 (100.0) [31/31]	0.490	0.381	<.001
Biopsy rate (%)	-	39.7 [25/63]	23.8 [15/63]			<.001
Asymptomatic (n=135)						
AUC	0.75 (0.68, 0.82)	0.66 (0.60, 0.73)	0.81 (0.74, 0.88)	0.073	0.155	<.001
Sensitivity (%)	67.8 (57.8, 77.8) [57/84]	97.6 (94.4, 100.0) [82/84]	97.6 (94.4, 100.0) [82/84]	<.001	<.001	-
Specificity (%)	82.3 (71.9, 92.8) [42/51]	35.3 (22.2, 48.4) [18/51]	64.7 (51.6, 77.8) [33/51]	<.001	0.010	<.001
PPV (%)	86.4 (78.0, 94.6) [57/66]	71.3 (63.0, 79.6) [82/115]	82.0 (74.5, 89.5) [82/100]	<.001	0.273	<.001
NPV (%)	60.8 (49.3, 72.4) [42/69]	90.0 (76.9, 100.0) [18/20]	94.3 (86.6, 100.0) [33/35]	<.001	<.001	0.084
Biopsy rate (%)	-	68.2 [92/135]	57.0 [77/135]			<.001
Asymptomatic and Mammography normal/benign (n=57)						
AUC	0.70 (0.56, 0.85)	0.70 (0.63, 0.78)	0.85 (0.77, 0.92)	0.976	0.058	<.001
Sensitivity (%)	60.0 (35.2, 84.8) [9/15]	100.0 (100.0) [15/15]	100.0 (100.0) [15/15]	-	-	-
Specificity (%)	80.9 (69.1, 92.8) [34/42]	40.5 (25.6, 55.3) [17/42]	69.1 (55.1, 83.0) [29/42]	<.001	0.090	<.001
PPV (%)	52.9 (29.2, 76.7) [9/17]	37.5 (22.5, 52.5) [15/40]	53.6 (35.1, 72.0) [15/28]	0.302	0.764	<.001
NPV (%)	85.0 (73.9, 96.0) [6/40]	100.0 (100.0) [17/17]	100.0 (100.0) [29/29]	0.302	0.764	<.001
Biopsy rate (%)		38.6 [22/57]	21.1 [12/57]			<.001

Note- Data in parentheses are 95% confidence intervals, and data in brackets are numerators and denominators.

AUC = area under the curve; PPV = positive predictive value; NPV = negative predictive value; US = ultrasound.

*For combined assessment, BI-RADS category 3 or 4A masses are downgraded to BI-RADS category 2 or 3 if Mastocheck® value is less than 0.0668.

Discussion

We showed that the hypothetical addition of Mastocheck® value to breast US assessment could improve its AUC, specificity, and PPV. According to our results, combined Mastocheck® and breast US assessment significantly increased the AUC of breast US alone from 0.67 (95% CI, 0.61, 0.73) to 0.81 (95% CI 0.75, 0.88; $P < .001$), specificity from 35.6% (95% CI: 23.4, 47.8) to 64.4% (95% CI: 52.2, 76.6; $P < .001$) without a loss in sensitivity and 44.8% (17/38) of false-positive findings could be eliminated. Diagnostic performance improvement was consistently observed in asymptomatic women and in women with negative or benign findings on mammography, mimicking the screening setting. Our study showed that Mastocheck® has the potential to be integrated with breast US to better triage women who really need or can spare breast biopsy or short-term follow-up in both screening and diagnostic settings.

Our combined diagnostic approach utilizing blood-based liquid biopsy with breast US in both screening and diagnostic settings showed the potential to eliminate unnecessary biopsies and reduce the short-term follow-up rate for BI-RADS category 3 or 4A lesions with increased specificity and PPV. Early breast cancer detection is important for improved breast cancer-related mortality and morbidity outcomes, but avoidance of unnecessary biopsies and short-term follow-up induced by breast US should also be considered, because they increase medical burden and patient anxiety. Thus, various studies have attempted to overcome the limitations of breast US [17] using elastography or color Doppler US in addition to grayscale B-mode US images [37, 38]. One study showed that the addition of elastography and color Doppler US to grayscale B-mode US improved the specificity from 27.0–76.4% and PPV from 8.9–23.2%, while avoiding 67.7% unnecessary biopsies for non-malignant lesions and without loss in sensitivity [37]. However, these additional US techniques are operator-dependent [38]. Recently, the use of deep learning models [39–41] has also been proposed to reduce unnecessary biopsies and increase cost-effectiveness. All these attempts used advanced techniques of US or B-mode morphologic features of mass. Our study is unique in that we evaluated not only morphological characteristics but also blood-based proteomics biomarker information.

A number of serum protein markers in breast cancer have been identified, but they were used primarily for monitoring response to therapy in patients with advanced breast cancer and only a few were suitable for screening [28–30]. Mastocheck®, a liquid biopsy biomarker developed for early breast cancer detection using proteomics technology, can be detected in trace amounts of 1 μL (0.001 cc) plasma and is validated to be specific for breast cancer [32]. Lee et al. used liquid chromatography-mass spectrometry to quantify levels of three proteins and input them into the algorithm to predict the presence of breast cancer: model=0.604× [CAH1] + 7.575× [NCHL1] − 0.523× [APOC1] [33]. Using this model, the sensitivity, specificity, and AUC were 78.7%, 78.7%, and 0.83, respectively. The performance of the model was improved in patients with stage I and II disease, with an AUC of 0.85. Thus, they suggested that their algorithm with higher performance in early stage breast cancers was beneficial for detecting breast cancers in the asymptomatic phase [33]. In a prior study from the same study group, total of 460 plasma samples, 228 from breast cancer patients and 232 from healthy controls, was used to validate their algorithm and reported diagnostic accuracy with AUC of 0.88 [31]. Mastocheck® was also useful in patients with dense breasts, showing increased sensitivity from 59.2% (mammography alone) to 93.0% (mammography and Mastocheck®) [32, 33]. Similarly, our subgroup analysis shows significant improvement in the diagnostic performance of US by the addition of Mastocheck® values in asymptomatic women. This shows the potential of Mastocheck® as a supplemental screening tool for breast cancer. In the era of precision medicine, understanding the utility of liquid biopsy will be necessary, and radiologists should provide more personalized management beyond imaging with liquid biopsy as another cost-effective mean of screening and diagnosis of breast cancer [19].

In our study, Mastocheck® showed lower sensitivity (70.8%) and AUC (0.74) and especially low NPV (46.9%) compared to previous studies [31–33]. This may be explained by different characteristics and proportions of breast cancer in the study population. In the verification and validation settings of prior study [33], the proportion of cancer was approximately 50.0% (80 breast cancer patients with 80 healthy controls in verification and 100 breast cancer patients with 100 healthy controls in validation); however, in this study, the proportion of cancer was 75.1% (178/237), which is also higher than that of previous two studies (60.0%, 183/305 and 50.9%, 575/1,129) [31, 32]. It is well known that the predictive value of a diagnostic test depends on the prevalence of the disease; NPV decreases and PPV increases with increased prevalence of the disease [42]. Indeed, in our subgroup analyses for mammographically occult or asymptomatic women with relatively lower disease prevalence, the NPV of Mastocheck® was higher than that of the entire cohort (60.8%-85.0%). Larger prospective studies are needed to assess the diagnostic performance more accurately and further research on Mastocheck® is needed to investigate the best cut-off value to achieve high sensitivity along with high NPV to result in fewer false negatives.

In our study, although there was no loss in sensitivity by using the definition of test-positivity of BI-RADS category 3 or higher on breast US, five cancers that were initially assessed as BI-RADS category 4A were downgraded to BI-RADS category 3 due to negative Mastocheck® result. They were all node-negative early stage breast cancers (two 0.5-cm and 0.2-cm invasive ductal carcinomas, two 0.5-cm invasive lobular carcinomas and one ductal carcinoma in situ). Four patients with invasive cancers showed masses measuring less than 1.0 cm on breast US and one patient diagnosed with ductal carcinoma in situ had suspicious calcification on mammography. Further validation of liquid biopsy should assess its influence on clinical management regarding the risk of delayed diagnosis of breast cancer and the benefit of avoiding false-positive findings and unnecessary biopsies.

Our study has several limitations. It is a single-institution, retrospective study, and selection bias is inevitable. Due to our study design aimed to select patients who had blood samples and breast US, our study cohort was a cancer-enriched population and composed of patients collected during long study period. In addition, we were only able to interpret static US images. Reader variability in breast US interpretation was not

considered. In combined assessment of breast US and Mastocheck®, we used our own criteria and it should be validated in the larger population. A larger prospective study with a variety of study populations is required to assess the clinical efficacy of Mastocheck®.

In conclusion, noninvasive proteomics biomarker assay, Mastocheck®, is an effective tool that can be used concomitantly with breast US for both detection of breast cancer and avoidance of false-positive findings and unnecessary biopsies. Our combined diagnostic approach with Mastocheck® and breast US has yielded promising results, and the outlook remains optimistic. It is certainly possible that Mastocheck® plays an even greater role in breast cancer clinics and improves diagnostic confidence. Further, larger prospective studies evaluating performance in high-risk and broader populations are needed to more accurately assess its utility and expand the use of this personalized assay.

Declarations

Disclosure of Potential Conflicts of Interest

Hong-Kyu Kim and Yumi Kim have unlisted stocks of Berits Inc.

Dong-Young Noh report conflict of interest as he became CEO of Berits Inc. since March 2021.

Wonshik Han reports being a member on the board of directors of and holding stock and ownership interests at DCGen, Co., Ltd., not relevant to this study.

Su Min Ha and Jung Min Chang have no conflict of interest.

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Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent This retrospective study was approved by the institutional review board, and the informed consent requirement was waived.

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Figures

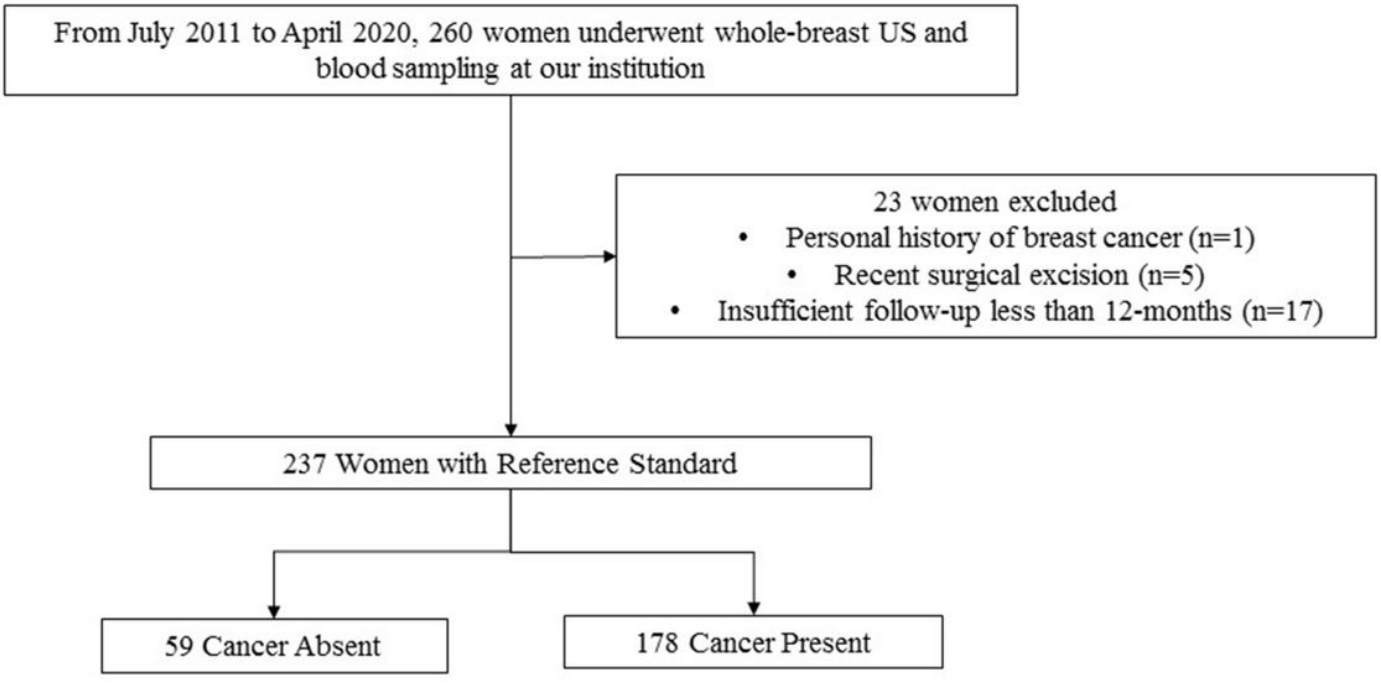

Figure 1

Flowchart of study population

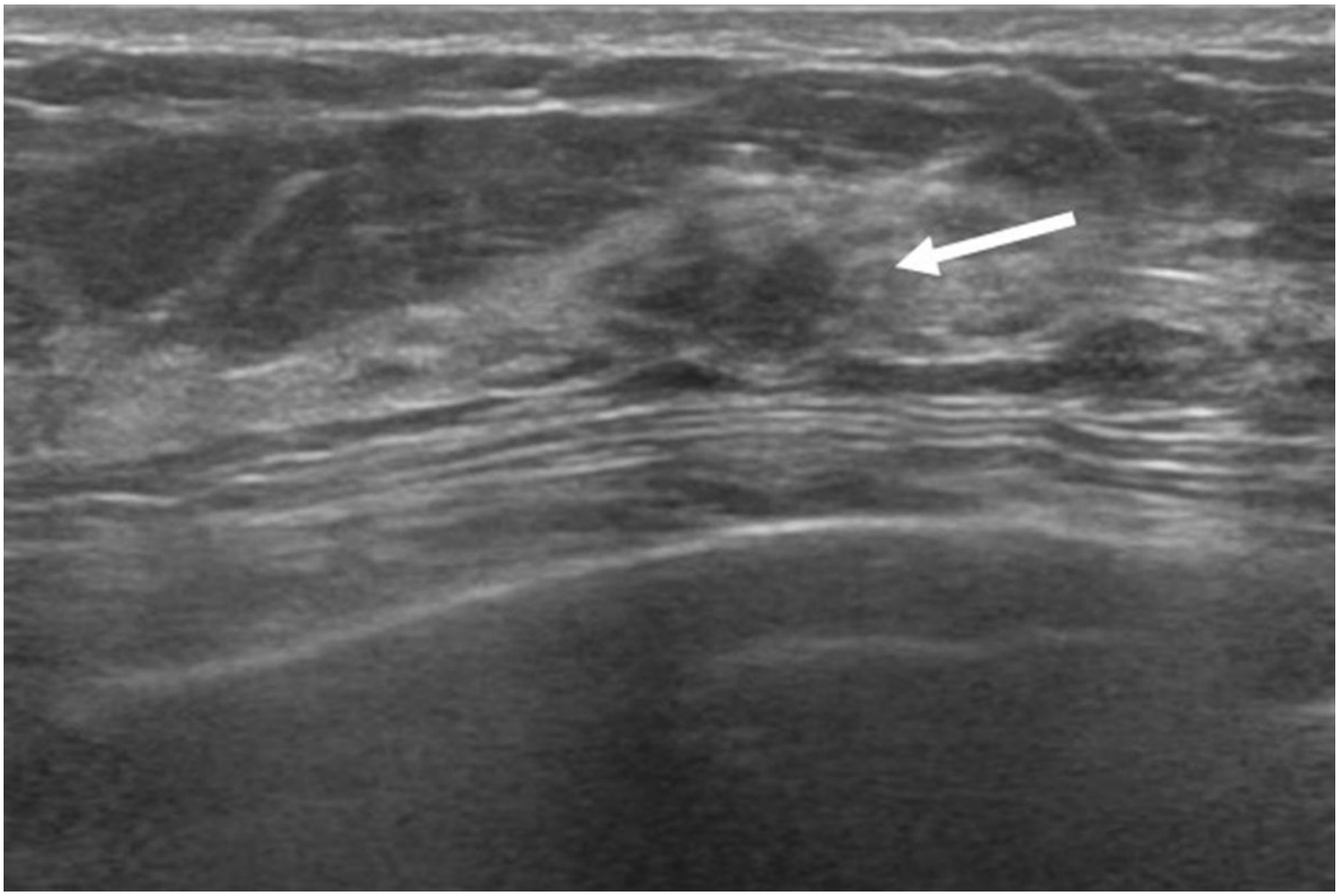

Figure 2

A 55-year-old woman with ductal carcinoma in situ, high grade. Breast ultrasound shows a hypoechoic mass with indistinct margin, classified as BI-RADS category 4A (arrow). The Mastocheck® value is 1.7754. Combined assessment is BI-RADS category 4A and surgical result shows high grade ductal carcinoma in situ, true-positive case.

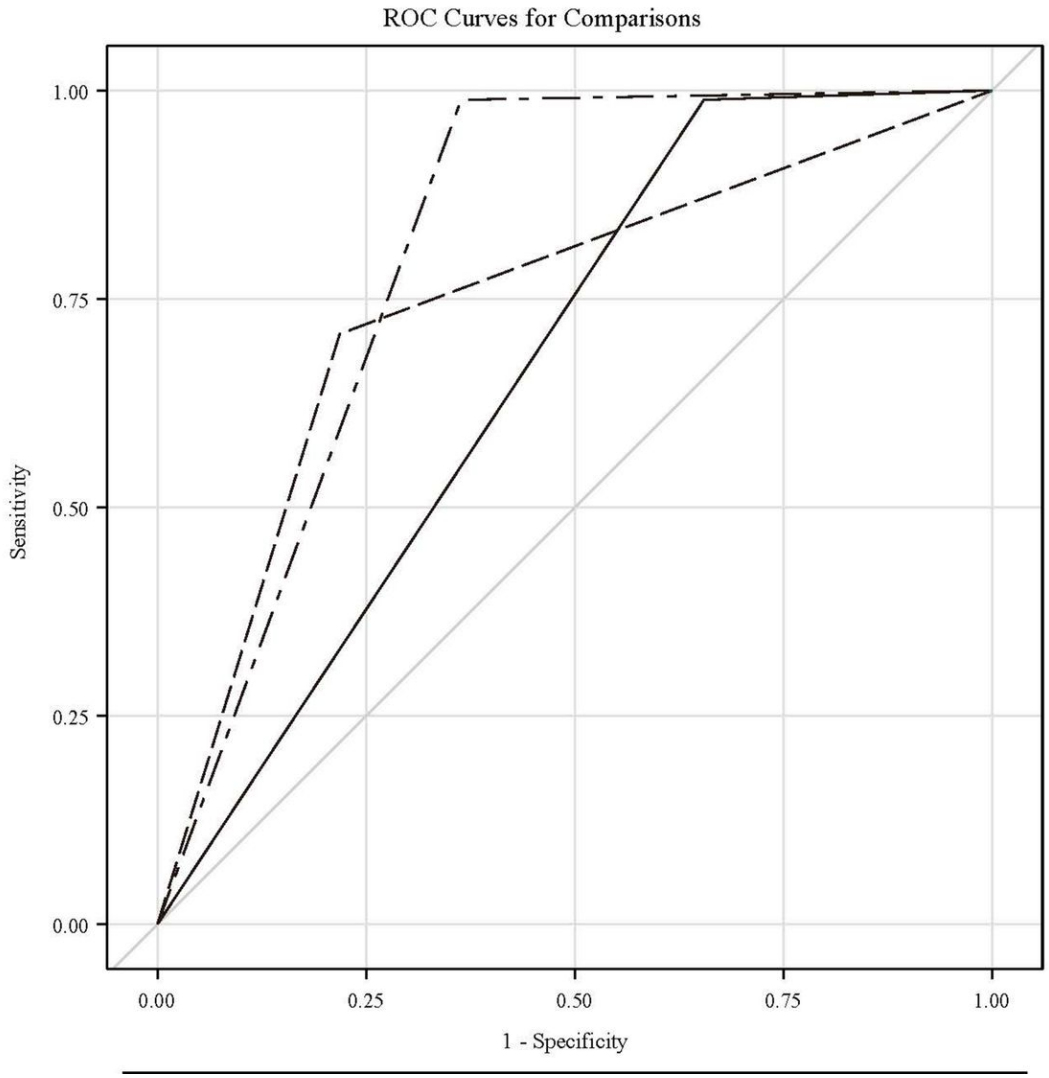

Figure 3

Receiver Operating Characteristic curve for Mastocheck®, breast ultrasound, and Mastocheck® combined with breast ultrasound. The AUC of breast ultrasound (solid line) is 0.67 (95% confidence interval [CI]: 0.61, 0.73) and Mastocheck® (dashed line) is 0.74 (95% CI, 0.68, 0.81). AUC is increased to 0.81 (95% CI: 0.75, 0.88) (P <. 001) by addition of Mastocheck® to breast ultrasound (dot-dashed line).

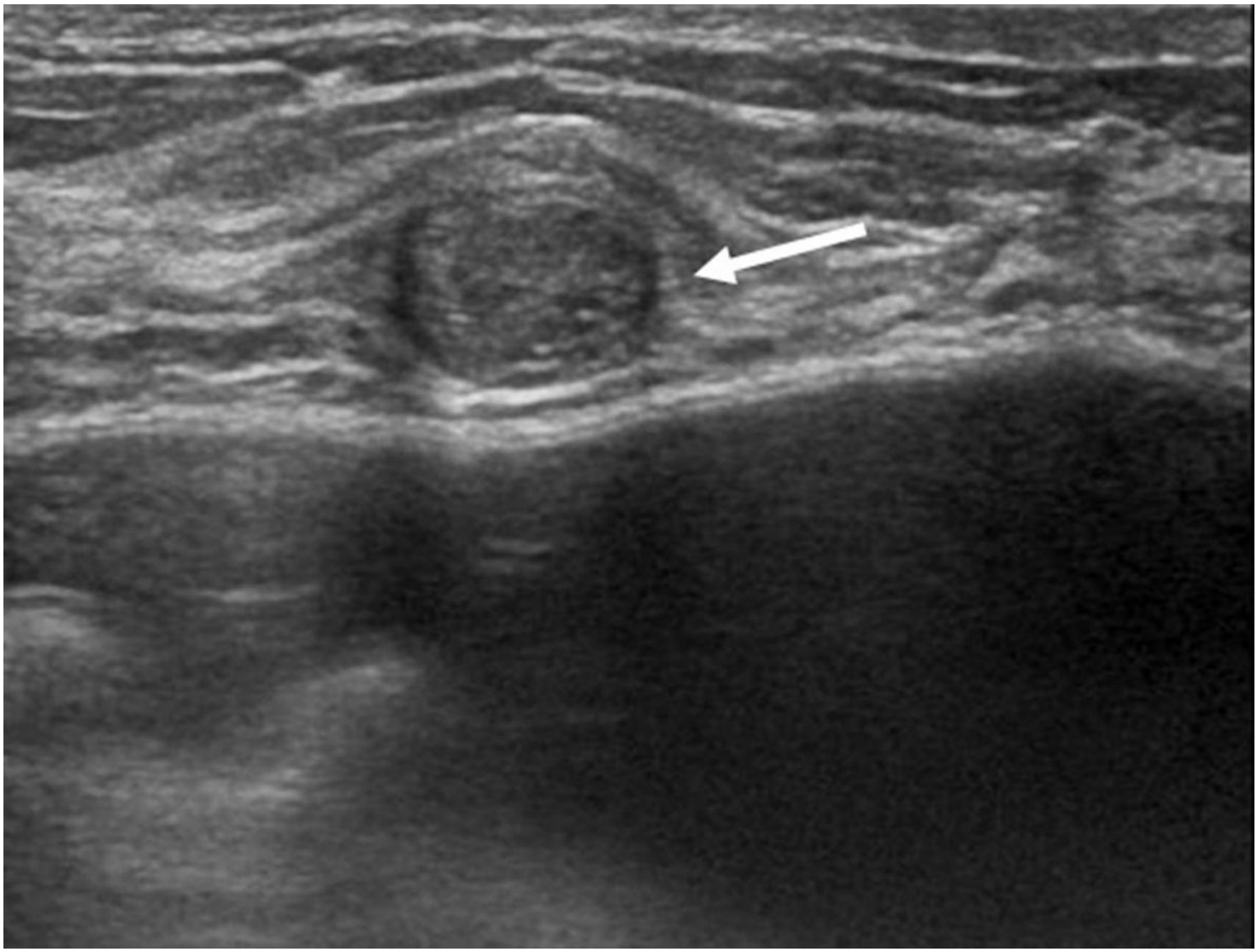

Figure 4

A 50-year-old woman with fibroadenoma. Breast ultrasound shows an oval mass with mostly circumscribed margin, classified as BI-RAD category 4A (arrow). The Mastocheck® value is 0.02 and combined assessment correctly downgraded the lesion to BI-RADS category 3, thus the biopsy can be avoided.

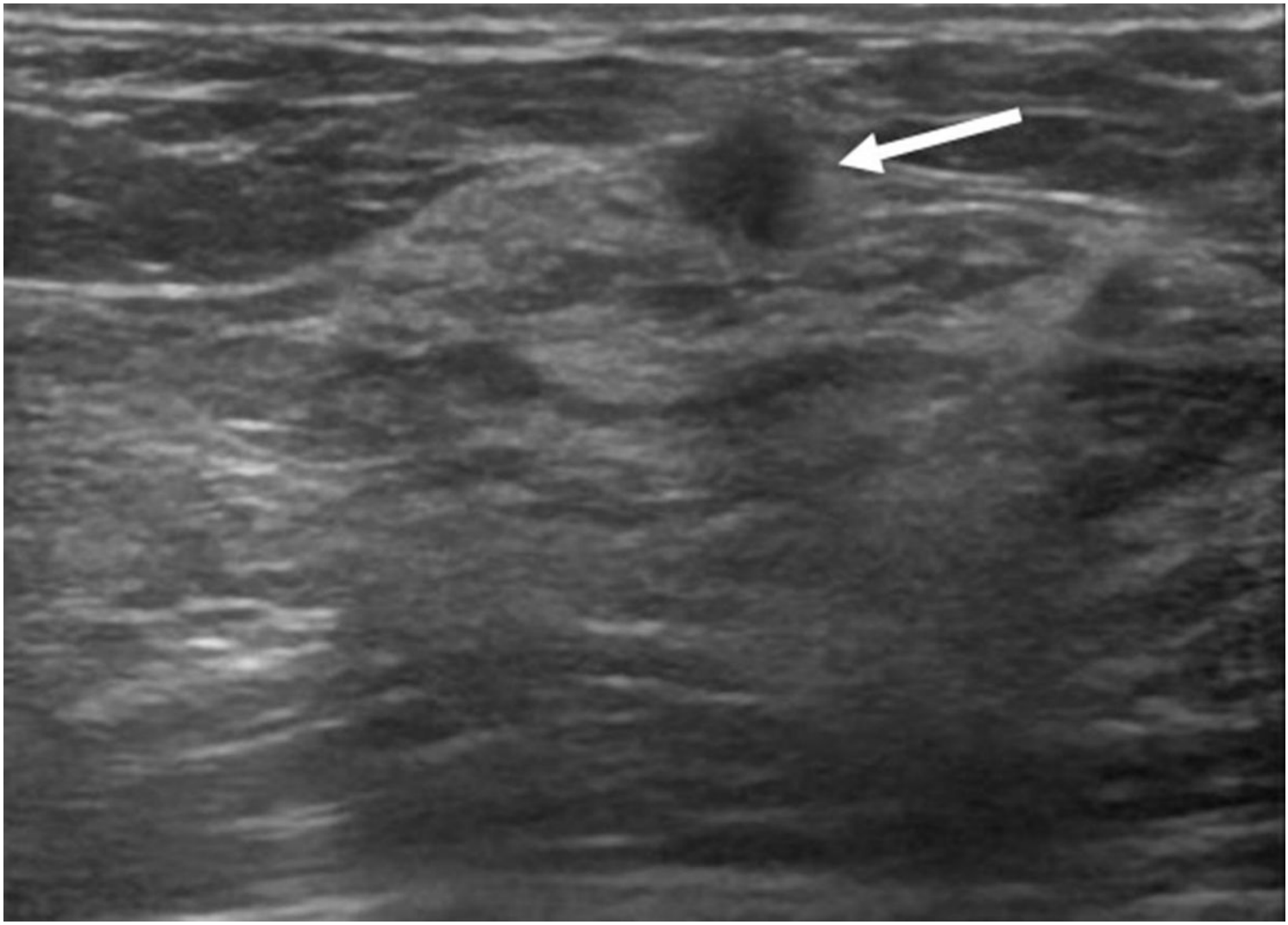

Figure 5

A 63-year-old woman invasive ductal carcinoma. Breast ultrasound shows a hypoechoic mass with indistinct margin, classified as BI-RADS category 4A (arrow). The Mastocheck® value result is -0.38. Combined assessment downgraded the lesion to BI-RAD category 3 and surgical result shows invasive ductal carcinoma grade I, estrogen receptor positive, progesterone receptor positive, and human epidermal growth factor receptor 2 negative.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplemtaryFig1.jpg
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