Original Article

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대한영상의학회지

Contrast-Enhanced Ultrasound Parameters in Breast Cancer: Correlations with Prognostic Factors

유방암의 조영 증강 초음파 매개변수: 예후 인자와의 관계

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Purpose To correlate the value of contrast-enhanced ultrasound (CEUS) with prognostic factors of breast cancer.

Materials and Methods 24 breast cancer patients were evaluated with CEUS. As a quantitative analysis, the peak enhancement (PE), wash-in and wash-out area under curve (WiWoAUC), wash-in rate (WiR) and wash-out rate, rise time, fall time, mean transit time, time to peak, and wash-in perfusion index (WiPI) were measured. As a qualitative analysis, the enhancement patterns were evaluated. Pathologic prognostic factors, including histologic grade, hormonal receptors and Ki-67 proliferative index were assessed by immunohistochemistry. Correlation of quantitative and qualitative parameters of CEUS with prognostic factors was assessed.

Results We found that the quantitative CEUS values (PE, WiWoAUC, and WiPI) of estrogen receptor (ER) positive breast cancer were higher than those of ER negative counterpart (all $p < 0.05$). Lower quantitative CEUS values (PE, WiWoAUC, WiR, and WiPI) were found in triple-negative cancer (TNC) than those of non-TNC (all $p < 0.05$). No CEUS parameter showed significant difference in distinguishing histologic grade (all $p > 0.05$).

Conclusion The CEUS parameters were helpful in predicting prognostic factors, such as ER positivity or triple negativity. However, they could not predict the histologic grade.

Index terms Breast Neoplasms; Ultrasonography; Prognosis; Receptors, Progesterone; Receptors, Estrogen; Immunohistochemistry

INTRODUCTION

Angiogenesis plays a primary role in the natural course of breast cancer; it is strongly related with both growth and metastasis of tumors (1-3). For this reason, when it comes to predicting the prognosis of breast cancer, the evaluation of the tumor vascularization is important. The problem is that breast masses show a distinctive pattern of vascularization; small tumor vessels with a slow flow. For this reason, detection, characterization, and quantification of the blood flow is challenging (4).

Contrast-enhanced ultrasound (CEUS) is a technique based on the detection of blood supply in and around the lesion (5). By using first generation contrast media, CEUS enhances the Doppler signal, the display of blood flow, and reveals perfusion characteristics in tumors (6, 7). In combination with high-resolution ultrasound (US) equipment and improved US contrast media, CEUS has been of much aid in studies of tumor angiogenesis at the microcirculation level (6).

So far, a lot of literature has been published focusing on the differential diagnosis between benign and malignant breast masses using CEUS (8-10). There have been quite a few reports on the correlation of qualitative CEUS parameters with prognostic factors as well (11, 12). Recent studies showed that quantitative CEUS parameters can distinguish the status of some pathological prognostic factors in breast invasive ductal carcinomas (IDCs) (13, 14). Furthermore, some reported that quantified CEUS parameters may even provide predictive value for high-grade malignancy (15).

Various studies have been conducted regarding the correlation between CEUS parameters and prognostic factors, but few studies have correlated both the quantitative and qualitative CEUS parameters with the prognostic factors simultaneously (16). Thus, we aimed to correlate both the quantitative and qualitative CEUS parameters with the prognostic factors of breast invasive carcinomas and explore the value of CEUS in predicting the immuno-histochemical biomarkers and histologic grade.

MATERIALS AND METHODS

PATIENTS

We prospectively enrolled 41 patients who had invasive carcinomas of the breast between January 2014 and July 2016 at Seoul St. Mary's hospital, the Catholic University of Korea. The study was about early prediction of the response of breast cancer to neoadjuvant chemotherapy (NAC) using CEUS, and written informed consent was obtained from all the study participants. The results of the study were published (17).

This is a retrospective study to examine the relationship between CEUS parameters and prognostic factors of invasive breast cancer using previously performed CEUS data. In early 2018, the Institutional Review Boards at the Catholic University of Korea approved this retrospective study (KC18RESI0031). All 41 advanced breast cancer patients scheduled for NAC were evaluated with baseline CEUS. Some biopsy data were missing, including histologic grade and immune histochemical results in 17 patients, and those 17 patients were excluded. Finally, 24 patients aged 46.3 ± 17 y (mean ± standard deviation) (range: 33–67 y) were in-

cluded in this study.

CONVENTIONAL US AND CEUS

Conventional US images were acquired with the Philips iU22 Color Ultrasound system (Philips, Bothell, WA, USA), which was equipped with a linear probe L12–5 MHz. Pulse-inversion harmonic imaging with a mechanical index of 0.07 was used. All US examinations were performed by two experienced radiologists with more than 5 and 10 years of experience in breast imaging.

All patients were asked to lie in the supine position with both upper limbs pointing up to fully expose their breasts and bilateral axillae. Examinations were performed as followed: First, conventional B-mode US was performed to scan the breast lesion and to observe US features such as the diameter, boundary, and echoing features. During B-mode US scanning, maximal tumor diameter was chosen as the ideal plane for CEUS. Second, the US system was then switched to contrast mode for CEUS examination. The parameters remained the same during the examination so as to ensure high-quality imaging. Second-generation microbubble contrast agent (sulfur hexafluoride, SonoVue®; Bracco, Milano, Italy) was injected through a 21-G catheter via a septum of subclavian Port-a-Cath system (PORT-A-CATH® II; Smiths Medical, St. Paul, MN, USA). Contrast agent (2.4 mL) was injected manually as a bolus over 2 seconds followed by a flush of 10 cc saline solution. The entire CEUS process was recorded for each patient from the time of injection, until no apparent agent was observed. CEUS studies were saved in the US hard disc system (native data).

IMAGING ANALYSIS

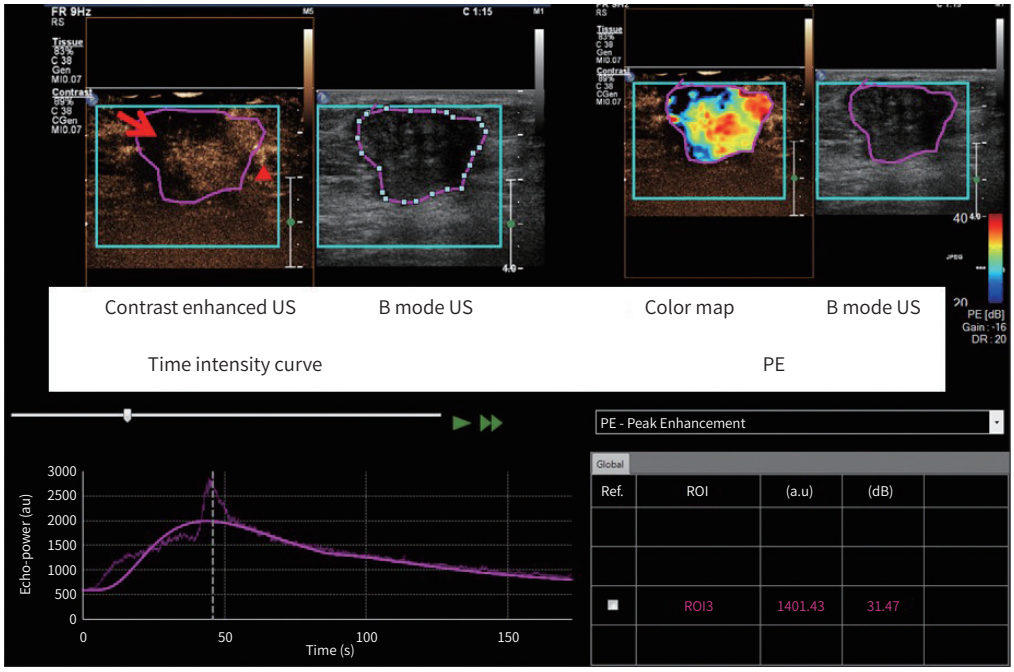
Image analysis was performed by two approach method; contrast-enhancement kinetic analysis (quantitative analysis) and contrast-enhancement pattern analysis (qualitative analysis). The data of CEUS studies were transferred to PC for further quantitative analyses with advanced US quantification software (VueBox®; Bracco, Suisse SA, Geneva, Switzerland).

Two radiologists drew regions of interest (ROI) on gray-scale US images. It was automatically copied and displayed in the contrast-enhanced images at the same time. When the ROI of the CEUS image and gray-scale image differ, the gray-scale image is used as a reference. The ROI is presented in Fig. 1. The ROI (purple) covered the entire tumor boundary of gray-scale US, avoiding surrounding parenchyma. The computer-assisted program allowed quantitative analysis such as acquisition of time (s)/echo power (arbitrary unit, au) curves. The time-intensity curve (TIC) was acquired and analyzed. The relevant parameters were calculated, including peak enhancement (PE) (au), which is the maximum enhanced strength of the curve; time to peak (s), which is the time required to reach PE; wash-in area under the curve (WiAUC) (au); wash-out area under the curve (WoAUC) (au); wash-in and wash-out area under the curve (WiWoAUC) (au); wash-in rate (WiR) (au), which is the maximum slope for microbubbles of contrast agent to enter into the tumor; wash-out rate (WoR) (au); rise time (RT) (s), which is the time from 10% to 90% of PE; fall time (s); mean transit time (s), which is the average time of contrast agent in tumor; and wash-in perfusion index (WiPI, WiAUC/rise time). The TIC is presented in Fig. 2.

As a qualitative analysis, enhancement patterns were classified as internal homogeneity,

perfusion defects, crab claw-like patterns, and the scope of the lesion (9-16). The internal homogeneity was assessed and classified as homogeneous or heterogeneous enhancement. Homogeneous enhancement was defined as diffuse and uniform enhancement of the lesion. Heterogeneous enhancement was defined as non-uniform with areas of variable signal intensity. The presence or absence of perfusion defect was noted. The presence or absence of the lesions showing crab claw-shape enhancement in the early phase was noted. The crab claw-shape enhancement was characterized by radial vessels, with small spiculated vessels

Fig. 1. An example of imaging analysis with advanced US quantification software (VueBox®). B mode US image shows ROI (purple line) drawn manually covering the entire tumor. In all cases, the depth was set to 4 cm. Static image of contrast enhanced US at the PE shows heterogeneous enhancement, perfusion defect (red arrow) and crab-claw enhancement (red arrowhead) at the periphery. Color map with PE shows variable enhancement in the tumor. Time-intensity curve is shown. And the PE value is shown (about 1401 au).
PE = peak enhancement, ROI = regions of interest, US = ultrasound



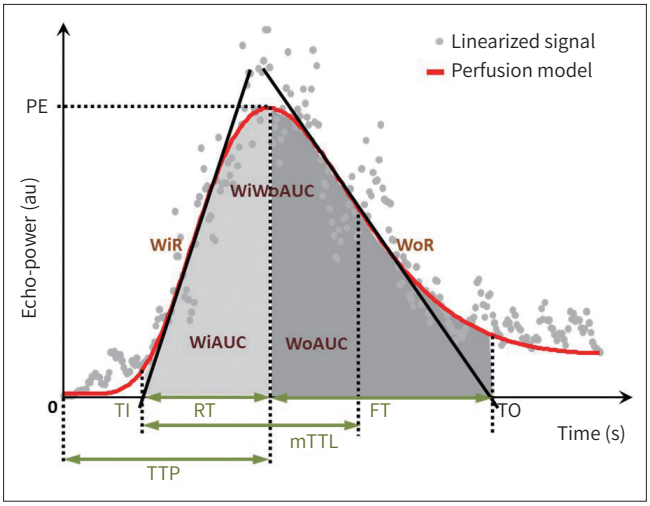


Fig. 2. Quantitative time-to-intensity curve parameters.
FT = fall time, mTTL = mean transit time, PE = peak enhancement, RT = rise time, TTP = time to peak, WiAUC = wash-in area under curve, WiR = wash-in rate, WiWoAUC = wash-in and wash-out area under the curve, WoAUC = wash-out area under curve, WoR = wash-out rate

commonly seen in the periphery region, and the scope of the lesion is noted; which is identical or larger than B-mode scale (purple line; ROI) (Fig. 1).

HISTOPATHOLOGICAL ANALYSIS

Pathological specimens obtained by means of US guided needle biopsy were analyzed by a breast pathologist with 10 years of experience in breast pathology. Histological grading of IDC was based on tubular structure, nuclear pleomorphism, and mitotic count according to the modified criteria of Bloom and Richardson, which is a widely accepted tumor grading system (18). The grades ranged from I–III; a score of 3–5 was considered grade I, 6–7 was grade II, and 8–9 was grade III. Immuno-histochemical staining was used to assess the following prognostic indicators: estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67. ERs and PRs were categorized as positive (> 1%) by stained nuclei in cancer cells on 10 high-power fields in accordance with recent studies (19, 20). The intensity of HER2 expression was semi-quantitatively scored as 0, 1+, 2+, or 3+. Tumors with a 3+ score were classified as HER2-positive and those with scores of 0 or 1+ as HER2-negative (21). Gene amplification was used to determine HER2 status for tumors with a 2+ score. Ki-67 status was expressed in terms of the percentage of positive cells, with a threshold of 14% drawn for positivity (22). Molecular subtypes of breast cancer were immunohistochemically classified as luminal (ER positive or PR positive or both), HER2 positive and triple negative type (negative for ER, PR and HER2). In particular, the ER positivity and triple negativity are noted in this study.

STATISTICAL ANALYSIS

Statistical analyses were performed using a software package (version 9.3; SAS Institute, Cary, NC, USA). Descriptive continuous variables are presented as mean ± standard deviation, and were analyzed using the Student's t-test, and Wilcoxon rank sum test. The differences in enhancement patterns between breast lesions were evaluated by the χ^2 test or Fisher exact test. For each analysis, a *p* value of less than 0.05 was considered significant.

RESULTS

HISTOPATHOLOGICAL ANALYSIS

Of all 24 invasive breast carcinomas, 8 (33.3%) were poorly differentiated (grade III), 16 (66.7%) were moderately (grade II) and well differentiated (grade I). There were 14 (58.3%) ER-positive, 11 (45.8%) PR-positive, 9 (37.5%) HER2-positive and 21 (87.5%) Ki-67-positive breast invasive carcinomas. There were 15 (62.5%) luminal type (luminal A and B), 4 (16.7%) HER2 positive type, and 5 (20.8%) triple negative type breast invasive carcinomas. There were 14 (58.3%) ER-positive, and 10 (41.7%) ER-negative type breast invasive carcinomas. There were 5 (20.8%) triple-negative cancer (TNC), and 19 (79.2%) non-TNC.

CORRELATION BETWEEN CEUS PARAMETERS AND PATHOLOGICAL PROGNOSTIC FACTORS

The descriptive statistics of the CEUS quantitative parameters on ROI in different patholog-

Table 1. Associations of Quantitative CEUS Parameters on ROI with Prognostic Factors of Breast Cancer

	Histologic Grade			ER			PR		
	I/II (*n* = 16)	III (*n* = 8)	*p*-Value (mean)	Negative (*n* = 10)	Positive (*n* = 14)	*p*-Value	Negative (*n* = 13)	Positive (*n* = 11)	*p*-Value
PE (au)									
Mean ± SD	1694.60 ± 1267.84	1174.16 ± 697.01	0.45	976.92 ± 583.97	1909.84 ± 1265.23	0.04	1148.63 ± 716.13	1961.34 ± 1373.57	0.17
Median (range; min–max)	1184.63 (205.89–4630.00)	1199.98 (260.29–2331.02)		991.71 (205.89–1845.31)	1710.78 (260.29–4630.00)		1076.78 (205.89–2331.02)	1380.57 (701.05–4630.00)	
WiAUC (au)									
Mean ± SD	37994.27 ± 25023.90	24773.43 ± 14320.35	0.21	21522.37 ± 14352.96	42205.15 ± 23876.65	0.02	26038.39 ± 17224.18	42508.80 ± 25659.79	0.11
Median (range)	27811.29 (4395.90–91280.77)	23064.85 (6494.66–51698.82)		20218.56 (4395.90–52366.53)	40115.59 (6494.66–91280.77)		22777.04 (4395.90–52366.53)	29529.25 (13749.37–91280.77)	
WoAUC (au)									
Mean ± SD	85683.08 ± 54995.16	65398.31 ± 50770.78	0.32	49128.67 ± 38133.53	100202.07 ± 53566.31	0.02	65671.80 ± 50038.83	94580.21 ± 55298.53	0.21
Median (range)	74854.48 (5941.13–199435.80)	45399.55 (18462.44–168747.90)		42795.02 (5941.13–127104.70)	93482.84 (18462.44–199435.80)		48907.16 (5941.13–168747.90)	88803.22 (22987.15–199435.80)	
WiWoAUC (au)									
Mean ± SD	123677.35 ± 79078.32	90171.75 ± 64782.60	0.29	70651.04 ± 52234.35	142407.23 ± 75739.51	0.02	91710.18 ± 66818.69	137089.02 ± 79573.28	0.17
Median (range)	102665.77 (10337.03–272441.60)	68464.395 (24957.10–220446.70)		64074.76 (10337.03–179471.20)	139022 (24957.10–272441.60)		75254.41 (10337.03–220446.70)	118332.50 (36736.52–272441.60)	
WiR (au)									
Mean ± SD	76.62 ± 61.76	63.07 ± 45.71	0.79	45.81 ± 31.15	90.89 ± 63.40	0.06	56.11 ± 39.96	91.01 ± 68.13	0.21
Median (range)	57.98 (7.67–208.94)	56.58 (12.47–141.09)		47.08 (7.67–110.39)	78.22 (12.47–208.94)		53.14 (7.67–141.09)	75.74 (21.66–208.94)	
WoR (au)									
Mean ± SD	25.45 ± 28.93	15.31 ± 8.45	0.70	14.37 ± 7.75	27.58 ± 30.44	0.34	14.38 ± 8.14	31.17 ± 33.37	0.23
Median (range)	14.88 (4.86–105.35)	16.41 (2.34–26.74)		16.01 (4.38–26.74)	17.11 (2.34–105.35)		17.21 (2.34–26.74)	14.81 (7.16–105.35)	
RT (s)									
Mean ± SD	35.44 ± 5.27	33.38 ± 6.05	0.19	33.81 ± 6.35	35.44 ± 4.93	0.21	34.35 ± 5.64	35.24 ± 5.56	0.65
Median (range)	34.59 (25.75–44.98)	31.25 (28.55–47.17)		31.55 (27.56–47.17)	35.16 (25.75–44.98)		33.63 (27.56–47.17)	34.36 (25.75–44.98)	
TTP (s)									
Mean ± SD	52.02 ± 14.24	44.71 ± 10.36	0.24	53.10 ± 15.99	47.08 ± 10.97	0.26	49.09 ± 15.05	50.17 ± 11.62	0.57
Median (range)	49.26 (36.54–90.95)	43.27 (33.45–60.33)		51.32 (33.44–90.95)	45.37 (35.06–77.45)		48.01 (33.45–90.95)	50.36 (35.22–77.45)	
WiPI (au/s)									
Mean ± SD	1101.68 ± 805.20	784.93 ± 488.66	0.53	636.88 ± 392.18	1252.68 ± 800.74	0.03	762.59 ± 489.16	1272.06 ± 866.82	0.17
Median (range)	799.05 (126.23–2927.54)	762.54 (177.22–1627.81)		654.76 (126.23–1239.86)	1146.82 (177.22–2927.54)		677.39 (126.23–1627.81)	949.56 (437.96–2927.54)	

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Table 1. Associations of Quantitative CEUS Parameters on ROI with Prognostic Factors of Breast Cancer (Continued)

	HER2			Ki-67			Subtype		
	Negative (*n* = 15)	Positive (*n* = 9)	*p*-Value	< 14% (*n* = 3)	≥ 14% (*n* = 21)	*p*-Value	Non TNC (*n* = 19)	TNC (*n* = 5)	*p*-Value
PE (au)									
Mean ± SD	1590.20 ± 1365.26	1405.99 ± 577.79	0.86	1683.09 ± 814.14	1497.98 ± 1173.48	0.51	1748.39 ± 1136.74	657.50 ± 496.75	0.02
Median (range)	1137.55 (205.89–4630.00)	1231.71 (701.05–2195.17)		2109.80 (744.29–2195.17)	1137.55 (205.89–4630.00)		1380.57 (260.29–4630.00)	489.51 (205.89–1372.35)	
WiAUC (au)									
Mean ± SD	34082.44 ± 26524.01	32762.14 ± 15417.24	0.95	42896.76 ± 19696.58	32257.41 ± 23116.67	0.40	38681.49 ± 22379.09	14229.50 ± 9735.77	0.02
Median (range)	26347.25 (4395.90–91280.77)	28129.32 (13749.37–52366.53)		52047.91 (20289.33–56353.04)	26347.25 (4395.90–91280.77)		29529.25 (6494.66–91280.77)	15184.84 (4395.90–26347.25)	
WoAUC (au)									
Mean ± SD	80254.70 ± 60609.39	76699.46 ± 41989.84	0.91	96662.26 ± 40806.98	76387.09 ± 55315.34	0.40	91405.79 ± 52235.63	31481.14 ± 23858.45	0.02
Median (range)	62213.78 (5941.13–199435.80)	77265.87 (22987.15–127104.70)		116769.40 (49703.59–123513.80)	62213.78 (5941.13–199435.80)		88803.22 (18462.44–199435.80)	35187.52 (5941.13–59155.45)	
WiWoAUC (au)									
Mean ± SD	114337.15 ± 85715.97	109461.59 ± 57195.55	0.95	139559.04 ± 60258.36	108644.50 ± 77264.05	0.40	130087.29 ± 73254.28	45710.63 ± 33140.27	0.02
Median (range)	84608.92 (10337.03–272441.60)	105395.20 (36736.52–179471.20)		173122.50 (69992.93–175561.70)	84608.92 (10337.03–272441.60)		118332.50 (24957.10–272441.60)	50372.36 (10337.03–79810.13)	
WiR (au)									
Mean ± SD	77.55 ± 67.96	63.03 ± 29.55	0.91	66.05 ± 32.98	72.97 ± 59.38	0.81	82.98 ± 57.45	30.76 ± 25.59	0.04
Median (range)	53.15 (7.67–208.94)	62.80 (21.66–110.39)		75.74 (29.31–93.11)	53.15 (7.67–208.94)		69.32 (12.47–208.94)	16.32 (7.67–63.46)	
WoR (au)									
Mean ± SD	24.40 ± 30.66	18.19 ± 5.35	0.41	19.75 ± 10.95	22.41 ± 25.84	0.56	25.15 ± 26.24	10.39 ± 9.39	0.08
Median (range)	11.43 (2.34 ±105.35)	18.01 (9.77–26.98)		25.13 (7.16–26.98)	14.81 (2.34–105.35)		18.01 (2.34–105.35)	6.08 (4.39–26.74)	
RT (s)									
Mean ± SD	34.33 ± 5.59	35.47 ± 5.59	0.73	39.59 ± 2.85	34.07 ± 5.47	0.08	34.87 ± 5.06	34.33 ± 7.64	0.63
Median (range)	34.11 (25.75–47.17)	33.74 (28.55–44.98)		41.06 (36.31–41.41)	33.63 (25.75–47.17)		34.11 (25.75–44.98)	31.70 (27.56–47.17)	
TTP (s)									
Mean ± SD	48.78 ± 14.04	50.94 ± 12.70	0.73	52.20 ± 5.23	49.21 ± 14.14	0.40	47.62 ± 10.55	57.07 ± 20.73	0.33
Median (range)	48.17 (35.06–90.95)	48.01 (33.45–77.45)		50.82 (47.81–57.99)	48.01 (33.45–90.95)		47.81 (33.45–77.45)	49.35 (36.54–90.95)	
WiPI (au)/s									
Mean ± SD	1041.09 ± 870.86	921.11 ± 392.61	0.86	1096.20 ± 522.63	981.80 ± 753.36	0.51	1145.98 ± 725.29	426.52 ± 321.74	0.02
Median (range)	764.42 (126.23–2927.54)	833.69 (437.96–1433.53)		1360.89 (494.17–1433.53)	764.42 (126.23–2927.54)		949.56 (177.22–2927.54)	321.92 (126.23–867.20)	

CEUS = contrast-enhanced ultrasound, ER = estrogen receptor, HER2 = human epidermal growth factor receptor 2, PE = peak enhancement, PR = progesterone receptor, ROI = regions of interest, RT= rise time, SD = standard deviation, TNC = triple-negative cancer, TTP = time to peak, WiAUC = wash-in area under the curve, WiPI = wash-in perfusion index, WiR = wash-in rate, WiWoAUC = wash-in and wash-out area under the curve, WoAUC = wash-out area under the curve, WoR = wash-out rate

Table 2. Associations of Qualitative CEUS Parameters on ROI with Prognostic Factors of Breast Cancer

	Histologic Grade			ER			PR		
	I or II (*n* = 16)	III (*n* = 8)	*p*-Value	Negative (*n* = 10)	Positive (*n* = 14)	*p*-Value	Negative (*n* = 13)	Positive (*n* = 11)	*p*-Value
Homogeneity									
Homogeneous	7 (70)	3 (30)	> 0.99	4 (40)	6 (60)	> 0.99	5 (50)	5 (50)	> 0.99
Heterogeneous	9 (64.3)	5 (35.7)		6 (42.9)	8 (57.1)		8 (57.1)	6 (42.9)	
Perfusion defect									
No	6 (75)	2 (25)	0.67	2 (25)	6 (75)	0.39	4 (50)	4 (50)	> 0.99
Yes	10 (62.5)	6 (37.5)		8 (50)	8 (50)		9 (56.2)	7 (43.8)	
Crab claw-like pattern									
No	6 (60)	4 (40)	0.67	6 (60)	4 (40)	0.21	7 (70)	3 (30)	0.24
Yes	10 (71.4)	4 (28.6)		4 (28.6)	10 (71.4)		6 (42.9)	8 (57.1)	
Scope of the lesion									
Identical	5 (83.3)	1 (16.7)	0.62	3 (50)	3 (50)	0.67	4 (66.7)	2 (33.3)	0.65
Larger than B-mode	11 (61.1)	7 (38.9)		7 (38.9)	11 (61.1)		9 (50)	9 (50)	
	HER2			Ki-67			Subtype		
	Negative (*n* = 15)	Positive (*n* = 9)	*p*-Value	< 14% (*n* = 3)	≥ 14% (*n* = 21)	*p*-Value	Non TNC (*n* = 19)	TNC (*n* = 5)	*p*-Value
Homogeneity									
Homogeneous	6 (60)	4 (40)	> 0.99	1 (10)	9 (90)	> 0.99	8 (80)	2 (20)	>0.99
Heterogeneous	9 (64.3)	5 (35.7)		2 (14.3)	12 (85.7)		11 (78.6)	3 (21.4)	
Perfusion defect									
No	6 (75)	2 (25)	0.66	1 (12.5)	7 (87.5)	> 0.99	7 (87.5)	1 (12.5)	0.63
Yes	9 (56.2)	7 (43.8)		2 (12.5)	14 (87.5)		12 (75)	4 (25)	
Crab claw-like pattern									
No	8 (80)	2 (20)	0.21	1 (10)	9 (90)	> 0.99	6 (60)	4 (40)	0.12
Yes	7 (50)	7 (50)		2 (14.3)	12 (85.7)		13 (92.9)	1 (7.1)	
Scope of the lesion									
Identical	5 (83.3)	1 (16.7)	0.35	1 (16.7)	5 (83.3)	> 0.99	4 (66.7)	2 (33.3)	0.57
Larger than B-mode	10 (55.6)	8 (44.4)		2 (11.1)	16 (88.9)		15 (83.3)	3 (16.7)	

CEUS = contrast-enhanced ultrasound, ER = estrogen receptor, HER2 = human epidermal growth factor receptor 2, PR = progesterone receptor, ROI = regions of interest, TNC = triple-negative cancer

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ical prognostic factors are summarized in Table 1 and the same for the qualitative parameters in Table 2.

Unlike previous research, we found a statistically significant difference showing that the quantitative CEUS values including PE, WiAUC, WoAUC, WiWoAUC, and WiPI of ER-positive breast cancer were higher than that of the ER-negative counterpart (individually, p = 0.04, 0.02, 0.02, 0.02, 0.03). Also, lower quantitative CEUS values including PE, WiAUC, WoAUC, WiWoAUC, WiR, and WiPI were found in TNC than that of non-TNC (individually, p = 0.02, 0.02, 0.02, 0.02, 0.04, 0.02).

Consistent with the previous studies, no quantitative CEUS parameters showed a significant difference between low grade (grade I, II) and high histologic grade (grade III), and neither did qualitative parameters (all $p > 0.05$). The qualitative CEUS parameters including enhancement patterns, perfusion defects, and the scope of tumors were not related to prognostic factors (all $p > 0.05$). The qualitative CEUS features of ER-positive breast cancer, especially crab claw-like enhancement pattern (neo angiogenesis) in this study showed a high percentage (71.4%), compared to that of ER-negative breast cancer (28.6%). The perfusion defects (tumor necrosis) of the ER-negative breast cancers showed a high percentage (80%). The qualitative CEUS features of TNC, especially perfusion defects in this study showed a high percentage (80%), compared to that of non-TNC counterpart (63.2%).

DISCUSSION

CEUS of the breast is a non-invasive method for evaluating the degree of tumor vascularization, which uses real blood pool contrast agents that cannot easily pass into intercellular

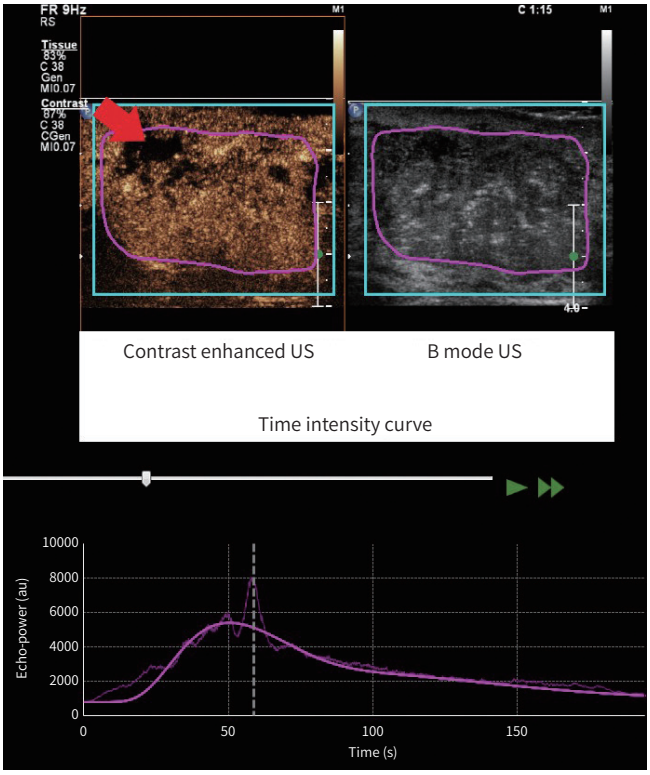

Fig. 3. A 45-year-old woman presented with invasive ductal carcinoma, ER-positive type. The tumor showed a relatively high wash-in area under curve value (91280.77 au) of region of interest (purple circle). Other tumor characteristics were as follows: low histologic grade; ER positive; progesterone receptor positive; human epidermal growth factor receptor 2 negative; Ki-67 ≥ 14%; luminal subtype. Gray-scale breast US image shows an irregular hypoechoic mass. Contrast-enhanced US image shows heterogeneously hyper-enhanced mass with a local perfusion defect (red arrow). The scope of the lesion is larger than that shown by gray-scale US. Time-intensity curve shows peak intensity value (about 4627 au).
ER = estrogen receptor, US = ultrasound

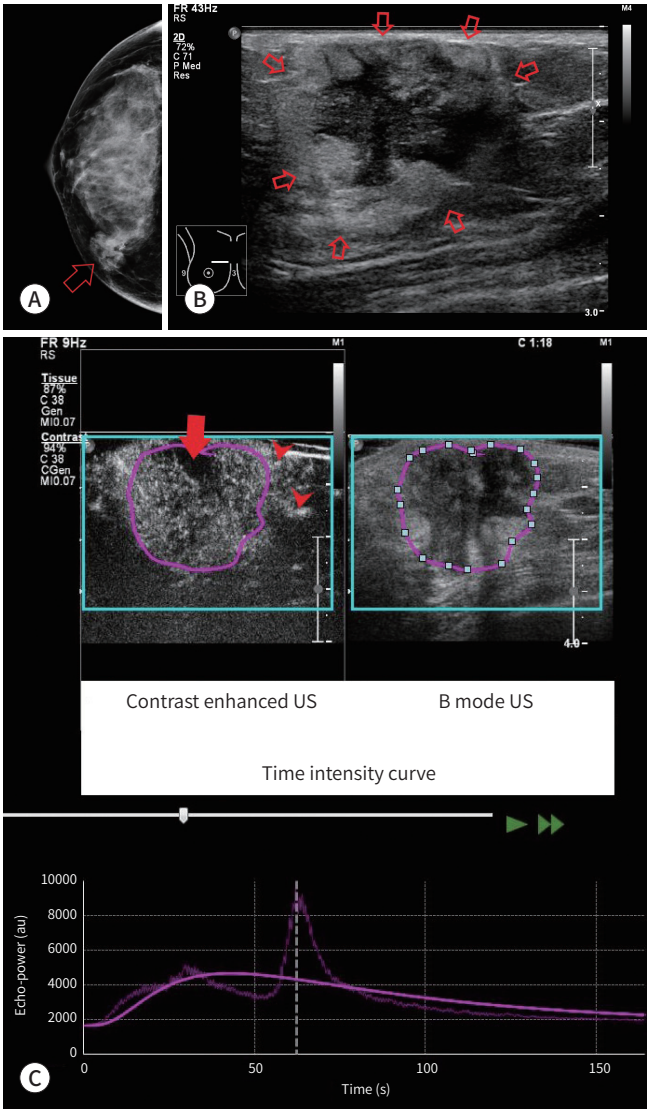

Fig. 4. A 44-year-old woman presented with Rt breast cancer, ER-positive type.
A. Mammography shows an indistinct oval equal density mass (arrow).
B. Gray-scale breast US image shows an oval indistinct heterogeneous mass (central hypoechoic and peripheral hyperechoic) (arrows).
C. Contrast-enhanced US image shows heterogeneously enhanced mass with a perfusion defect (red arrow) and crab-claw enhancement (red arrowheads) at the periphery. The scope of the lesion is slightly larger than it appears in the corresponding gray-scale US image. Time intensity curve shows peak intensity value (about 3164 au). The tumor showed a relatively high wash-in area under curve value (73005.78 au) of region of interest (purple circle). Other tumor characteristics were as follows: low histologic grade, ER positive, progesterone receptor positive, human epidermal growth factor receptor 2 negative, Ki-67 ≥ 14%, luminal subtype.
ER = estrogen receptor, US = ultrasound

spaces. Quantitative analysis with CEUS provides an objective and reproducible method for the evaluation of the degree of vascularization. Previous research has revealed that PE reflects the quantity of contrast agent microbubbles in the vascular bed of the lesion; while WiR reflects the early flow quantity and velocity during contrast agent perfusion. These two parameters are associated with the degree of vascularization. Different CEUS enhancement patterns (qualitative analysis) at different regions of breast cancer are primarily attributed to blood perfusion disparities (23). Previous studies have revealed that some quantitative or qualitative CEUS parameters showed statistically significant differences between breast IDCs with different pathological prognostic factors (11-16). One study of 100 IDC patients (78 ER-positive cancers, 18 HER2 positive cancers, 14 TNCs and 54 Ki-67 positive cancers) have showed that the PE, WiR, and WiWoAUC values were significantly greater in ER-negative than ER-positive cancers (15). In this study, we found that the quantitative CEUS values including PE, WiAUC, WoAUC, WiWoAUC, and WiPI of ER-positive breast invasive carcinomas were higher than that of ER-negative carcinomas, which is opposite to result of previous

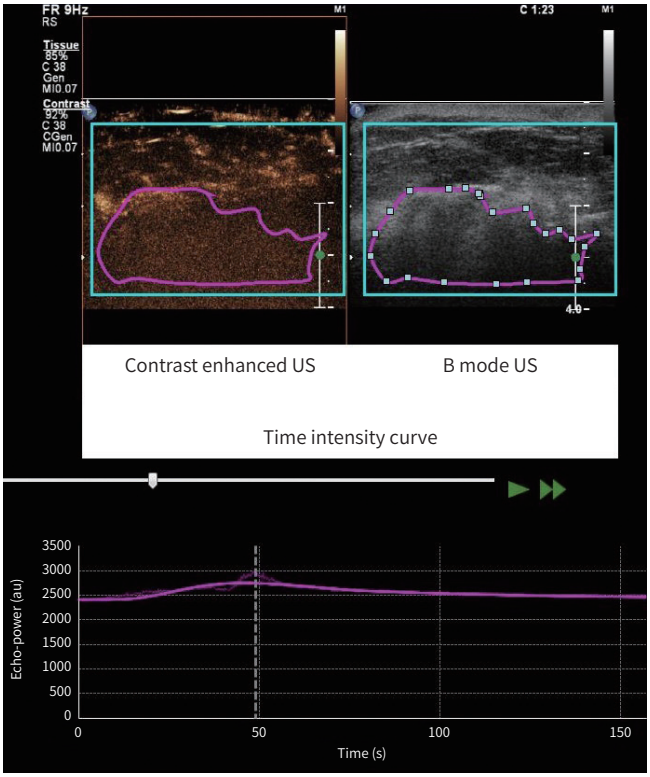

Fig. 5. A 54-year-old woman presented with multi-centric breast cancer; invasive ductal carcinoma, triple negative subtype. The tumor showed a relatively low wash-in area under the curve, wash-out area under the curve, wash-in and wash-out area under the curve, wash-in perfusion index values of region of interest (purple circle). Other tumor characteristics were as follows: low histologic grade; estrogen receptor negative; progesterone receptor negative; human epidermal growth factor receptor 2 negative; Ki-67 ≥ 14%. Gray-scale breast US image shows an irregular indistinct hypoechoic mass. Contrast-enhanced US image shows relatively faint and heterogeneous enhancement. Time-intensity curve shows low peak intensity value (about 337 au).
US = ultrasound

studies (all $p < 0.05$) (Fig. 3, 4). Several studies indicated that ER negative breast carcinomas commonly showed signs of central necrosis or fibrosis and the defects of vascular perfusion (11, 24, 25). An inverse association of micro-vessel density with ER expression has been reported (26). Given this fact, the result of this study, which is contrary to previous studies, may have been influenced by the fact that a relatively large number of atypical ER-positive patients with angiogenetic character (71.4% of crab claw-like enhancement pattern) were enrolled in this study (Fig. 4). The crab claw-like enhancement pattern is generally considered to be a relatively typical enhancement pattern for malignant tumors (27). This pattern, which is due to the presence of tortuous vessels, was clearly delineated in CEUS. Malignant cells secrete a variety of angiogenesis factors, particularly vascular endothelial growth factor, that promotes neo angiogenesis in and around the lesions. This may be the pathophysiological basis of the crab claw-like enhancement pattern. The low quantitative values of ER-negative cancers are also influenced by a high percentage of necrotic ER-negative cancer (80% of perfusion defect) in this study. Also, low quantitative CEUS values including PE, WiAUC, WoAUC, WiWoAUC, WiR, and WiPI were found in TNC than that of non-TNC (all $p < 0.05$). Previous studies have showed that the PE, WiR, WoR, and WiWoAUC were higher in TNCs than non-TNCs (15). Although this study revealed none of the quantitative and qualitative CEUS parameters showed a statistically significant difference according to the Ki-67 levels or HER2 positivity (all $p < 0.05$) (Fig. 5). Previous studies have revealed that the quantitative CEUS values including PE, WiR, WoR, and WiAUC are more frequently higher in malignant tumors with high Ki-67 levels than in those with low Ki-67 levels (15). Also, the other previous research of 102 IDC patients (70 ER-positive cancers, 62 PR-positive cancers, 89 HER2 positive cancers,

and 94 Ki-67 positive cancers) have showed that the quantitative CEUS parameters of RT with HER2 positive cancer was shorter than the negative ones (13). Positive expression of Ki-67 was considered to be an important marker of tumor cell proliferative activity (2). The expression of HER2 can also predict pathogenesis and progress in breast cancer, and its overexpression is closely related to distant metastasis (28). In this study, it is possible that a small number of Ki-67 positive (n = 21) or HER2 positive (n = 9) cancer patients resulted in different outcomes.

The qualitative CEUS features of TNC, especially perfusion defects in this study showed a high percentage (80%). A perfusion defect is an important index for evaluation. Malignant tumors grow faster than benign lesions, and neo angiogenesis and nutrition supply are relatively insufficient. Therefore, the local area of the tumor may become hypoxic and necrotic (29). Morphological studies of TNCs have shown a higher prevalence of the presence of necrosis (30). Previous studies have showed that the lesions of breast cancer were highly and heterogeneously enhanced sometimes with central defects, whose diameters were enlarged, and the edges became crab-like and blurry (9-16). Usually, a scope was extended in size because of the malignancy in IDCs due to carcinoma in situ component, located in the surrounding part of the lesion. In addition, adenosis surrounding malignant lesions may be hyper-vascular, which increases the scope in contrast-enhanced mode (31). However, the enhancement shape and enhancement margin of tumors are not related to prognostic factors (16). Consistent with previous research, our study revealed that none of the qualitative CEUS parameters including enhancement patterns, such as internal homogeneity, perfusion defects, crab claw-like enhancement patterns, and the scope of tumors showed statistical difference in distinguishing histologic grade or prognostic factors. According to this result, it may be inaccurate in predicting prognostic factors when quantitative and qualitative CEUS parameters are evaluated separately; both quantitative and qualitative parameters should be evaluated simultaneously to correctly predict the prognostic factors.

Our study had several limitations. First, this was a retrospective study, and the number of patients was relatively small. Second, this study was conducted in a single center, and this may have led to selection bias. Third, our study included only advanced breast cancer patients scheduled for NAC. Fourth, the patients we concentrated on had only the most common subtypes of IDC, while other types of breast cancer such as invasive lobular carcinoma or medullary carcinoma were not included.

In summary, while quantitative and qualitative CEUS parameters using ROI on the entire tumor area of gray-scale US were helpful in predicting prognostic factors, such as ER positivity or triple negativity in breast invasive carcinomas, such parameters could not predict the histologic grade. Further investigation needs to be conducted with larger sample sizes to ensure the reliability and accuracy.

Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

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유방암의 조영 증강 초음파 매개변수: 예후 인자와의 관계

이명은[1] · 김성헌[2*] · 강봉주[2] · 이윤주[3] · 김윤주[4]

목적 유방암 환자에서 조영 증강 초음파 변수 값이 유방암의 예후 인자와 어떤 상관관계를 갖는지 알아보고자 하였다.

대상과 방법 유방암 환자 24명의 조영 증강 초음파 검사를 시행하였다. 정량적 분석으로 최대 조영 증강, 조영 증강과 세척 곡선하면적, 조영 증강되고 사라지는 속도와 시간, 평균체류시간, 최고점 도달시간, 조영 증강되는 시간당 곡선하면적을 측정하였다. 정성적 분석으로 조영 증강 양식을 평가하였다. 병리학적 예후 인자로 조직학적 등급, 호르몬수용체, Ki-67 증식인자는 면역화학조직학적으로 분석되었다. 정성적, 정량적 조영 증강 초음파 매개변수와 예후 인자와의 연관성을 분석하였다.

결과 난포호르몬양성 유방암에서 정량적 조영 증강 초음파 매개변수 값(최대 조영 증강, 조영 증강과 세척 곡선하면적, 조영 증강되는 시간당 곡선하면적)이 난포호르몬음성 유방암의 경우보다 높았다(모든 $p < 0.05$). 삼중음성호르몬 유방암에서 정량적 조영 증강 초음파 매개변수 값(최대 조영 증강, 조영 증강과 세척 곡선하면적, 조영 증강속도, 조영 증강되는 시간당 곡선하면적)이 비삼중음성호르몬 유방암의 경우보다 낮았다(모든 $p < 0.05$). 조직학적 등급을 구분하는 데 있어서 유의하게 차이가 나는 조영 증강 초음파 매개변수 값은 없었다(모든 $p > 0.05$).

결론 조영 증강 초음파의 매개변수는 난포호르몬양성 또는 삼중호르몬음성 여부와 같은 유방암의 예후 인자를 예측하는데 있어서 도움이 될 수 있지만, 조직학적 등급은 예측할 수 없다.

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