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● *Original Contribution*

# EVALUATION OF MALIGNANCY GRADE OF BREAST CANCER USING PERFLUBUTANE-ENHANCED ULTRASONOGRAPHY

Norio Masumoto,* Takayuki Kadoya,* Ai Amioka,* Keiko Kajitani,* Hideo Shigematsu,* Akiko Emi,* Kazuo Matsuura,* Koji Arihiro,† and Morihito Okada*

*Department of Surgical Oncology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan; and †Department of Anatomical Pathology, Hiroshima University Hospital, Hiroshima, Japan

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**Abstract—Whether the contrast effects of perflubutane on contrast-enhanced ultrasonography can predict the malignancy grade of breast cancer is unknown. We analyzed associations between perfusion parameters created from time–intensity curves based on enhancement intensity and temporal changes in contrast-enhanced ultrasonography and clinicopathologic factors in 100 consecutive patients with invasive breast cancer. Values of perfusion parameters were significantly greater in estrogen receptor-negative than -positive tumors (peak intensity, $p = 0.0002$; ascending slope, $p = 0.006$; area under the curve, $p = 0.0006$). Variations in the peak intensity of Ki-67 were significantly correlated in all tumors ($r = 0.54, p < 0.0001$) and in luminal ($r = 0.43, p = 0.0002$), human epidermal growth factor receptor type 2-positive ($r = 0.47, p = 0.047$) and triple-negative ($r = 0.55, p = 0.043$) tumors. Perfusion parameters on contrast-enhanced ultrasonography can provide excellent predictive value for high-grade malignancy and might help to determine appropriate therapeutic strategies. (E-mail: morihito@hiroshima-u.ac.jp)** © **2016 World Federation for Ultrasound in Medicine & Biology.**

***Key Words:*** **Breast cancer, Ultrasonography, Time–intensity curve, Estrogen receptor, Molecular subtype.**

## INTRODUCTION

Ultrasonography (US) can help to differentiate benign from malignant mammary tumors (Costantini et al. 2006; Hong et al. 2005; Rahbar et al. 1999) and evaluate mass diameter. Flow imaging (Hieken et al. 2001; Sehgal et al. 2006) and elastography (Chang et al. 2011; Itoh et al. 2006) are also useful for differentiating benign from malignant tumors, and contrast-enhanced ultrasonography (CEUS) allows detailed real-time evaluation of hemodynamics in patients with mammary tumors. Benign and malignant tumors can be differentiated by CEUS using SonoVue (Balleyguier et al. 2009; Zhao et al. 2010). Contrast-enhanced magnetic resonance imaging is also very useful in differentiating benign from malignant tumors (Weinstein et al. 2010), to diagnose tumor spread (Amano et al. 2000) and to detect multicentric breast cancer (Mann et al. 2008). CEUS is equal to or better than plain US (Zhao et al. 2010) and perhaps even magnetic resonance imaging (Du et al. 2012) for differentiating benign from malignant tumors. Furthermore, CEUS using perflubutane is useful in identifying sentinel lymph nodes (Omoto et al. 2009) and predicting (Fujisawa et al. 2013) and determining (Cao et al. 2012) the effects of treatment that elicits a pathologic complete response after neoadjuvant chemotherapy (NAC). In addition, CEUS can be used to qualitatively and quantitatively evaluate changes in blood flow. Useful quantitative analyses reported to date include fractal/geometry (Amarteifio et al. 2013) and dispersion (Mischi et al. 2015) methods. Perfusion parameters quantitatively assessed by CEUS and the number of microvessels calculated by immunostaining correlate in breast cancer (Wan et al. 2012). We hope that quantitative evaluations will be applied to the morphologically distinctive features of tumors and the determination of outcomes of chemotherapy in the near future.

Characteristic molecular markers for breast cancer, including estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor type 2 (HER2) and Ki-67 proliferative index, are established prognostic factors and predictors of the outcomes of endocrine therapy and chemotherapy (Goldhirsch et al. 2011). Genetic profile analyses with microarrays have also been

Address correspondence to: Morihito Okada, Department of Surgical Oncology, Research Institute for Radiation Biology and Medicine, Hiroshima University, 1-2-3-Kasumi, Minami-ku, Hiroshima City, Hiroshima 734-0037, Japan. E-mail: morihito@hiroshima-u.ac.jp

used to classify the molecular subtypes of lesions that display distinctive gene expression profiles (Perou et al. 2000; Sørlie et al. 2001). Immunohistochemically stained phenotypes of ER, PR, tumor grade, HER2 and the Ki-67 proliferative index are used instead of molecular subtypes in actual clinical practice (Goldhirsch et al. 2013). Treatment strategies also differ because molecular subtypes of breast cancer react differently to systemic pharmacotherapy and have different vital prognoses (Cheang et al. 2009; Goldhirsch et al. 2011, 2013).

We investigated differences in contrast-effect intensity and contrast time in CEUS using perflubutane in patients with invasive ductal carcinoma (IDC). We also created time–intensity curves (TICs) of brightness and changes in the intensity of perflubutane contrast effects as the agent flowed into tumors. We then converted brightness into numerical values and investigated correlations between contrast-effect intensity and immunohistochemical markers.

## METHODS

### *Patient characteristics*

We prospectively enrolled 100 consecutive patients aged (mean ± standard deviation) aged 54.9 ± 13.1 y (range: 31–74 y) who had clinical stage I or II IDC of the breast between August 2012 and June 2014. NAC was allowed, and 28 patients received pre-operative NAC. All patients were treated by either mastectomy or breast-conserving surgery. The 72 patients who did not receive NAC were evaluated by pre-operative CEUS and by analysis of post-operative pathology specimens. The 28 patients who received NAC were evaluated by CEUS and by analysis of biopsy specimens obtained before starting NAC. Therefore, patients who received NAC were assessed by both pre-chemotherapy CEUS and pathologic specimen analysis. All patients were evaluated by CEUS, and pathologic specimens were analyzed before starting chemotherapy.

We evaluated nuclear grade (NG), Ki-67 proliferative index and ER as well as HER2 status in all patients for whom complete clinicopathologic information was available (Table 1). The institutional review boards at Hiroshima University approved this study, and all patients provided written informed consent to participate.

Table 1. Clinical and pathologic characteristics of patients with breast cancer

| Factor | *n* |
|---|---|
| Mean age ± SD (y) | 54.9 ± 13.1 |
| <50 y | 47 |
| ≥50 y | 53 |
| Clinical T | |
| T1 | 53 |
| T2 | 47 |
| Clinical N | |
| N0 | 78 |
| N1 | 22 |
| Nuclear grade | |
| 1 | 8 |
| 2 | 32 |
| 3 | 60 |
| Ki67 | |
| <30 | 46 |
| ≥30 | 54 |
| Estrogen receptor | |
| Negative | 22 |
| Positive | 78 |
| HER2 | |
| Negative | 82 |
| Positive | 18 |
| Tumor subtype | |
| Luminal A | 31 |
| Luminal B | 37 |
| HER-2-positive | 18 |
| Triple-negative | 14 |

HER2 = human epidermal growth factor receptor 2.

### *Ultrasonography and contrast-enhanced ultrasonography*

Conventional US images were acquired using a HI VISION Ascendus (Hitachi–Aloka Medical, Mitaka, Tokyo, Japan) with a 5- to 13-MHz linear-array transducer, in longitudinal and transverse planes, from supine patients with raised arms. Lesions were measured, and representative images of the index cancer (lesion with the largest diameter) were acquired. Blood perfusion images acquired using CEUS with perflubutane microbubbles stabilized with a phosphatidylserine membrane (Daiichi Sankyo, Tokyo, Japan) were qualitatively and quantitatively assessed. CEUS proceeded using an EUP-L74 M 5- to 13-MHz linear-array transducer (Hitachi–Aloka Medical, Mitaka, Tokyo, Japan) and color wide-band pulse inversion, as well as coded-phase inversion harmonic US with a mechanical index of 0.23 at a single focus zone at the tumor depth. Other scanner settings on the ultrasound device included dynamic range (65) and gain (17). All patients received a bolus intravenous injection of 0.015 mL/kg perflubutane at the elbow, followed by a flush with 20 mL of saline. The largest area of the tumor (longest diameter) was assessed by CEUS. Tumors were continuously imaged for >50 s immediately after the saline injection. The quantitative acquisition time for kinetic analysis was 50 s. Ultrasound images and video clips were stored on a hard disk for subsequent analysis. Three surgeons who acquired the US images and two other physicians who assessed the images confirmed correspondence among the findings.

### *Setting regions of interest (tumor contours) on CEUS images*

The largest area of the tumor (longest diameter) was assessed by CEUS and the largest area was set as the region