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● *Original Contribution*

QUANTITATIVE ANALYSIS OF CONTRAST-ENHANCED ULTRASOUND IMAGING IN INVASIVE BREAST CANCER: A NOVEL TECHNIQUE TO OBTAIN HISTOPATHOLOGIC INFORMATION OF MICROVESSEL DENSITY

Naoko Mori,* Shunji Mugikura,* Shoki Takahashi,* Koichi Ito,† Chiaki Takasawa,* Li Li,* Minoru Miyashita,‡ Atsuko Kasajima,§ Yu Mori,¶ Takanori Ishida,‡ Tetsuya Kodama,‖ and Kei Takase*

*Department of Diagnostic Radiology, Tohoku University Graduate School of Medicine, Sendai, Japan; †Department of Information Science, Tohoku University Graduate School, Sendai, Japan; ‡Department of Surgical Oncology, Tohoku University Graduate School of Medicine, Sendai, Japan; §Department of Pathology, Tohoku University Graduate School of Medicine, Sendai, Japan; ¶Department of Orthopedic Surgery, Tohoku University Graduate School of Medicine, Sendai, Japan; and ‖Department of Biomedical Engineering, Tohoku University Graduate School, Sendai, Japan

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Abstract—We examined whether enhancement area ratios obtained by the new bubble detection method correlate with histologic microvessel density in invasive breast cancer. Forty consecutive patients with invasive breast cancer lesions underwent contrast-enhanced ultrasound. The ratio of enhanced area to manually segmented tumor area (enhancement area ratio) was obtained with the new method at peak and delayed phases (50–54, 55–59, 60–64 and 65–69 s). We also analyzed time–intensity curves to obtain peak intensity and area under curve. Enhancement area ratios in both peak and delayed phases (50–54, 55–59, 60–64 and 65–69 s) were significantly correlated with microvessel density ($r = 0.57, 0.62, 0.68, 0.61$ and 0.58; $p = 0.0001, <0.0001, <.0001, <.0001$ and 0.0001, respectively). In time–intensity curve analysis, peak intensity was significantly correlated ($r = 0.43, p = 0.0073$), whereas area under the curve was not ($r = 0.29, p = 0.0769$). Enhancement area ratios obtained by the new method were correlated with microvessel density in invasive breast cancer. (E-mail: naokomori7127@gmail.com) © **2016 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Ultrasonography, Contrast medium, Breast cancer, Microvessels, Quantitative evaluation.**

INTRODUCTION

Growth and metastasis of breast cancers depend on the growth of new blood vessels in and adjacent to the tumor (Carmeliet and Jain 2000; Uzzan et al. 2004). Microvessel density (MVD) is generally considered to be a measure of new blood vessel formation, and several studies have reported a significant relationship between MVD and risk of metastasis (Horak et al. 1992; Uzzan et al. 2004; Weidner and Gasparini 1994). In patients with breast cancer, high MVD is associated with shorter relapse-free survival and decreased overall survival for both node-negative (Toi et al. 1993; Uzzan et al. 2004; Van et al. 1993; Weidner et al. 1992) and node-positive (Nieto et al. 2007; Toi et al. 1993) disease.

Evaluation of MVD might be useful in predicting prognosis; several attempts have been made to evaluate MVD using pre-operative imaging. Dynamic contrast-enhanced magnetic resonance imaging (MRI) is one such modality in common clinical use (Huuse et al. 2012) and has been studied as a means of detecting differences in tumor vasculature using the enhancement effect. Although contrast-enhanced MRI can be used to characterize benign and malignant tumors, assessment of MVD by this method has yielded inconsistent results; one study of *in vivo* contrast-enhanced MRI reported a significant correlation between the pattern and rate of contrast uptake and histologic MVD (Buadu et al. 1996), whereas others did not (Hulka et al. 1995; Stomper et al. 1997; Teifke et al. 2006). This inconsistency may be ascribed to the fact that the administered MRI contrast agent does not remain entirely within intravascular spaces; a

Address correspondence to Naoko Mori, Department of Diagnostic Radiology, Tohoku University Graduate School of Medicine, Seiryo 1-1, Sendai, Miyagi 9808574, Japan. E-mail: naokomori7127@gmail.com

significant portion distributes into the surrounding extracellular spaces. Instead of contrast-enhanced MRI, microbubble contrast-enhanced ultrasound (CEUS) imaging has been tried for the evaluation of tumor vasculature or perfusion. Microbubbles remain solely intravascular in nature (Loveless et al. 2008; Szabó et al. 2013) because they have a diameter of 2–3 μm and cannot pass through the vascular endothelial interspace, enabling direct evaluation of the vasculature.

Several methods used to analyze the quantitative enhancement effect of CEUS imaging have been reported (Lassau et al. 2010), including simple subtraction of pre- and post-contrast enhanced US imaging (Lyshchik et al. 2007; Willmann et al. 2010), temporal maximum-intensity-projection imaging (Pysz et al. 2011; Wilson et al. 2008) and time–intensity curve analysis (TIC) after intravenous administration of microbubbles (Wan et al. 2012). Even though some of these methods are already available with US systems, problems impede their clinical use. Results of the subtraction method and maximum-intensity-projection imaging can be significantly skewed if patients move during the examination (Ito et al. 2015; Wilson et al. 2008); the subtraction method is not clinically available yet for this reason. Meanwhile, TIC analysis has been difficult to implement: When comparing parameters among patients, depths of target lesions and gain adjustment, both of which affect US signal intensity, should be identical in each patient (Ignee et al. 2010), which is clinically difficult to maintain.

In a new program, the bubble detection method, contrast agents of microbubbles in vessels are detected by assessing intensity variation in a pixel in the temporal axis, providing the proportion of vessels filled with contrast agents in a certain tissue volume (enhancement area ratio) (Ito et al. 2015). Contrast agents of microbubbles in vessels are observed as blinking signals in the temporal axis, as they are constantly in motion. Therefore, we can detect contrast agents in vessels by evaluating the intensity variation of a pixel in the temporal axis. We named our new method the new bubble detection method, because it enables detection of the microbubbles themselves. This method is considered to be more robust against subject motion during examination compared with conventional methods, and to require less strict scanning conditions with respect to target depth and gain adjustment.

We hypothesized that MVD could be accurately quantitated pre-operatively using the new bubble detection method. The purpose of our study was to evaluate whether enhancement area ratios at peak and delayed phases in the new bubble detection method correlate well with histologic MVD in invasive breast cancer.

METHODS

Patients

Our institutional review board approved this retrospective study and waived the informed consent requirement. Of 117 patients diagnosed with breast carcinoma by core needle biopsy histologically between August 2014 and December 2015, 66 patients with 66 lesions underwent pre-operative CEUS examination followed by mastectomy or lumpectomy. For 51 patients, CEUS examination was not performed because of the cost for medical care or the contraindication to contrast agent. Excluding patients treated with neoadjuvant therapy (n = 13), which likely caused some changes in MVD, and patients with ductal carcinoma *in situ* (DCIS) (n = 13), for which the clinical significance of MVD is unknown (Adler et al. 2012), 40 consecutive patients with 40 lesions of invasive breast cancer who underwent pre-operative CEUS examination were included in this study. All patients had unilateral breast carcinomas. In one patient with multiple lesions unilaterally in a breast, we evaluated the lesion with the largest diameter. All patients were female; their median age was 59 y (range: 37–76 y), and their median weight was 50 kg (range: 45–75 kg).

CEUS examination

The median interval between biopsy and CEUS was 69 d (range: 31–103 d). The ultrasound examinations were performed by two operators together (a radiologist and a sonographer with 6 and 20 y of experience in breast ultrasound, respectively). Gray-scale conventional US imaging was performed with an Aplio 500 (Toshiba Medical Systems, Tochigi, Japan) with a 10-MHz linear transducer. Color Doppler US was performed to evaluate intra- and extra-tumoral vascularity with low-velocity (4.2 cm/s) parameter settings and high gain. The plane with the most abundant vessels was selected with reference to color Doppler US. In cases without any Doppler signal or vascularity within the tumor, we selected the plane with the largest cross-sectional area of the tumor. CEUS was performed with the same unit, with a 5.5- to 7.5-MHz linear transducer, in dual-screen mode, which simultaneously displays images of both conventional B-mode and CEUS mode. We placed the transducer on the skin softly so as not to compress the tissue, thereby avoiding collapse of vascular lumens. The machine parameters were adjusted so that the mechanical index was 0.18–0.2, the frame rate was 17/s and the gain was 80–100 dB. No parameter was changed during the examination.

The contrast agent used in this study was Sonazoid (Daiichi Sankyo, Japan), a lipid-stabilized suspension of perflubutane microbubbles. The contents of each vial