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● *Original Contribution*

CONTRAST-ENHANCED ULTRASOUND CHARACTERISTICS OF BREAST CANCER: CORRELATION WITH PROGNOSTIC FACTORS

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Abstract—The purpose of the work described here was to investigate the correlation of contrast-enhanced ultrasound (CEUS) characteristics with prognostic factors in breast cancer. Forty-five consecutive breast cancer patients were studied with CEUS. All patients were diagnosed with invasive ductal carcinoma on the basis of biopsy or surgery results. Lack of blood perfusion of the tumor was identified in 2 cases; blood perfusion was observed in 43 cases. Enhancement was heterogeneous in 25 cases, and centripetal in 25 cases. A blood perfusion defect was present in 27 cases. Enhancement shape was irregular in 37 cases, margins were poorly defined in 34 cases, and penetrating vessels were present in 32 cases. Compared with the surrounding normal tissue, tumor tissue had faster rising times and times to peak and higher peak intensities and wash-in slopes; the differences between the two groups were statistically significant ($p < 0.05$). Compared with the interior of the tumor, the periphery had faster times to peak and higher peak intensities and wash-in slopes ($p < 0.05$). Heterogeneous enhancement, perfusion defect, centripetal enhancement and penetrating vessels were correlated with prognosis ($p < 0.05$). Overall, some CEUS characteristics of breast cancer were associated with prognostic factors that can predict breast cancer prognosis *in vivo*. (E-mail: cxl969@sina.com) **© 2014 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Ultrasonography, Contrast agent, Breast cancer, Prognostic factor.**

INTRODUCTION

The classic prognostic factors of breast cancer—axillary lymph node status, tumor diameter and histologic grade—have been used to predict breast cancer recurrence and overall survival. However, the same clinical manifestations and pathologic characteristics often lead to different prognoses; therefore, greater attention is being attached to tumor molecular markers. The expression of estrogen receptor (ER) and progesterone receptor (PR) in breast cancer can predict tumor prognosis and guide hormonal therapy. Negative ER expression does not respond well to hormonal therapy, but is sensitive to chemotherapy (Guarneri et al. 2006). Proto-oncogene c-erb-B2 expression is closely related to pathogenesis and progress in breast cancer, and its over-expression is related to lung, bone and brain metastases (Gown 2008; Kulka et al. 2009). Ki-67 is considered an important marker in the evaluation of tumor cell proliferation activity, which is significantly correlated with tumor histologic grade and lymph mode metastasis. The cancer suppressor gene p53 is very susceptible to mutation in tumors, participating in cell growth and regulation (Li et al. 2004). Positive expression of Ki-67 and p53 reflects the ability of tumor cells to infiltrate and metastasize and corresponds to a poor prognosis. Tumor prognostic factors can be obtained only in histologic specimens. It is necessary to develop a non-invasive examination tool that is well correlated with tumor prognostic factors and can evaluate tumor biologic behavior and prognosis *in vivo* before surgery.

With the rapid development of medical imaging, the application of contrast-enhanced ultrasound (CEUS) in the diagnosis of breast masses has evolved quickly in recent years. CEUS can compensate for the limitation of color Doppler in the diagnosis of tumor angiogenesis and push forward ultrasound as a medical technique usable from pure morphologic diagnosis to morphology and functional diagnosis. Currently, CEUS is employed less often in breast diseases than in abdominal diseases. Most investigators have focused on discrimination

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between benign and malignant masses; correlation of CEUS enhancement characteristics with prognostic factors has seldom been reported. The purpose of our study was to analyze the CEUS characteristics of breast cancer and investigate the correlation between CEUS characteristics and prognostic factors in breast cancer.

METHODS

Patient information

Forty-five consecutive patients seen from October 2010 to June 2012 were included in this retrospective study, which was approved by the ethics committee of our institution. All patients were informed of the possible complications of CEUS and signed an informed consent before the CEUS examination. Age ranged from 33 to 76 y, with a mean ± standard deviation of 50.3 ± 8.7 y. Tumor maximum diameter averaged 30.5 ± 18 mm (range: 9–86 mm). The inclusion criterion was the appearance of a suspected malignant tumor on conventional ultrasound or mammography examination (BI-RADS [Breast Imaging-Reporting and Data System] category 4–5). Patients were excluded if they (i) had a pulmonary heart disease or respiratory syndrome; (ii) had severe hepatic and renal dysfunction; or (iii) were pregnant or lactating. Results of ultrasound-guided core needle biopsy or surgical pathology were obtained after the examination.

Contrast-enhanced ultrasound examination

A Philips iU22 Color Ultrasound system (Philips, Bothell WA, USA) with Philips L9-3 and L5-1 probes was used in this study. The L5-1 probe was used for tumors larger than 40 mm. Machine parameters were adjusted so that the mechanical index was 0.06–0.08 and gain was 100–120 dB. The entire CEUS process was recorded for at least 3 min.

All CEUS imaging of breasts was performed by an experienced clinical ultrasound physician, with more than 5 y of experience in ultrasound breast examination. Before contrast imaging, we tried to select the maximum tumor diameter based on conventional ultrasound (US) as the ideal plane for CEUS. The selected plane included the lesion and its surrounding normal tissue (defined as within 10 mm of the apparent margin on conventional US), if possible. The contrast agent used in the study was SonoVue (Bracco, Milan, Italy). All patients received an elbow intravenous bolus injection of 4.8 mL SonoVue, followed by a flush with 5 mL of saline solution.

Contrast-enhanced ultrasound image analysis

Imaging analysis was completed independently by two professional ultrasound physicians (with 4 and 3 y of experience with CEUS, respectively). Before participating in the research, the US physicians were trained to judge based on the same imaging assessment standard. Both physicians were blinded to patients' clinical data. If they disagreed, another physician (with 5 y of experience with CEUS) re-evaluated the image until a consensus was obtained.

Imaging analysis, based on our clinical experience and the literature (Zhao et al. 2010), comprised CEUS enhancement characteristics and parameters of the time-intensity curve. The CEUS enhancement characteristics evaluated were internal homogeneity, enhancement order, blood perfusion defect, enhancement shape, enhancement margin and penetrating vessels. Internal homogeneity was classified into four categories according to the distribution of the enhanced areas of the lesion: (1) no enhancement after contrast agent injection; (2) homogeneous enhancement visible across the whole lesion; (3) peripheral enhancement in or more confined to the lesion periphery; (4) heterogeneous enhancement. Enhancement order was classified as centripetal or centrifugal. Centripetal enhancement was defined as enhancement originating from the periphery of the lesion and developing centripetally. Centrifugal enhancement was enhancement originating from the center of the lesion and developing centrifugally. Enhancement margins were either well defined (>50% of the lesion circumference was clearly visible) or poorly defined (lesion was indistinct, 50% of the lesion circumference was clearly visible). Presence or absence of a perfusion defect was noted. Enhancement shape was evaluated as irregular or regular, and the penetrating vessels as absent or present.

The parameters of the time-intensity curve, obtained with Philips built-in analysis software (QLAB), were rising time (RT), time to peak (TTP), peak intensity (PI), wash-in slope (WIS) and mean transit time (MTT). A region of interest was selected in the area with the most rapid and strongest enhancement. Three regions of interest were selected for each lesion, and the mean value for each parameter was selected as the final value.

Immuno-histochemistry examination

The SP Reagent Kit from Fuzhou Manxin Biotechnology Development (Fuzhou, China) was used to carry out SP immuno-histochemical examination. Expression of ER, PR and p53 was considered positive when distinctive staining was observed in cell nuclei, whereas expression of c-erb-B2 was considered positive when distinctive staining was observed in the cell membrane or cytoplasm. The cutoff point for ER and PR positivity was 10%. Expression of c-erb-B2 was considered positive when distinctive membranous staining was observed in almost all tumor cells; it was considered negative when weak to moderate complete membrane staining was observed in more than 10% of tumor cells. Positive p53 expression was considered present in any case with well-defined