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Three-dimensional contrast enhanced ultrasound score and dynamic contrast-enhanced magnetic resonance imaging score in evaluating breast tumor angiogenesis: Correlation with biological factors

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ABSTRACT

Objective: To explore the clinical value of three-dimensional contrast enhanced ultrasound (3D-CEUS) and dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) score systems in evaluating breast tumor angiogenesis by comparing their diagnostic efficacy and correlation with biological factors.
Methods: 3D-CEUS was performed in 183 patients with breast tumors by Esaote Mylab90 with SonoVue (Bracco, Italy), DCE-MRI was performed on a dedicated breast magnetic resonance imaging (DBMRI) system (Aurora Dedicated Breast MRI Systems, USA) with a dedicated breast coil. 3D-CEUS and DCE-MRI score systems were created based on tumor perfusion and vascular characteristics. Microvessel density (MVD), vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMP-2, MMP-9) expression were measured by immunohistochemistry.
Results: Pathological results showed 35 benign and 148 malignant breast tumors. MVD ($P = 0.000$, $r = 0.76$), VEGF ($P = 0.000$, $r = 0.55$), MMP-2 ($P = 0.000$, $r = 0.39$) and MMP-9 ($P = 0.000$, $r = 0.41$) expression were all significantly different between benignity and malignancy. Regarding 3D-CEUS 4 points as cutoff value, the sensitivity, specificity and accuracy were 85.1%, 94.3% and 86.9%, respectively, and correlated well with MVD ($P = 0.000$, $r = 0.50$), VEGF ($P = 0.000$, $r = 0.50$), MMP-2 ($P = 0.000$, $r = 0.50$) and MMP-9 ($P = 0.000$, $r = 0.66$). Taking DCE-MRI 5 points as cutoff value, the sensitivity, specificity and accuracy were 86.5%, 94.3% and 88.0%, respectively and also correlated well with MVD ($P = 0.000$, $r = 0.52$), VEGF ($P = 0.000$, $r = 0.44$), MMP-2 ($P = 0.000$, $r = 0.42$) and MMP-9 ($P = 0.000$, $r = 0.35$).
Conclusions: 3D-CEUS score system displays inspiring diagnostic performance and good agreement with DCE-MRI scoring. Moreover, both score systems correlate well with MVD, VEGF, MMP-2 and MMP-9 expression, and thus have great potentials in tumor angiogenesis evaluation.

1. Introduction

Tumor angiogenesis played a central role in local tumor growth, invasion and distant metastasis, which was first confirmed in breast cancer [1]. Neovasculature of malignant tumor was entirely different from benign tumor both in morphology and hemodynamics. Therefore, the characteristics of tumor angiogenesis are of paramount importance in differentiation between benign and malignant lesions. Currently, breast tumor angiogenesis could be assessed by contrast-enhanced ultrasound (CEUS), dynamic contrast-enhanced computed tomography(CT) and dynamic contrast-enhanced magnetic resonance imaging

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(DCE-MRI), which are all able to quantify tumor perfusion, blood volume and permeability in capillary level, and further display tumor characteristics in hemodynamics for their better spatial and temporal resolution [2]. Compared with CT or MR contrast agent, microbubble ultrasound contrast agents are truly blood pool imaging agent which would not diffuse into cell gap, and thereafter greatly improves the sensitivity of blood flow detection in low velocity, accurately reflects breast tumor perfusion, and contributes a lot to breast tumor differentiation [3]. Combining the advantages of CEUS and 3D-US, three-dimensional contrast enhanced ultrasound (3D-CEUS) can objectively evaluate tumor vascularity by reconstruction of stereoscopic images. In particular, with high resolution and bilateral breast imaging, breast MRI is superior to other methods in detection of multi-center and multi-focal lesion for various sequence post-processing and three-dimensional reconstruction. Plenty of studies have demonstrated that the sensitivity and accuracy of DCE-MRI were significantly higher than mammography or conventional US by differential diagnosis in morphological and hemodynamic characteristics of enhanced breast tumor, whereas the specificity is somewhat lower [4,5]. Based on former results, we considered that for both 3D-CEUS and DCE-MRI, an objective score system is essential because just one characteristic cannot completely reflect the versatile vascular distribution. Simultaneously, microvessel density (MVD) has been accepted as the gold standard for evaluation of tumor angiogenesis [6]. Vascular endothelial growth factors (VEGF) have been found to be important cytokines in regulating endothelial cell proliferation and function development, which may also influence the expression of MVD [7], While MMPs family has been proved to be actively involved in biological changes of cancer, MMP-2, MMP-9 are especially closely related to tumor invasion and metastasis [8].

Therefore, our research aims at prospectively comparing the diagnostic efficacy and feasibility of 3D-CEUS score and DCE-MRI score and their association with these biological factors in clinical breast tumor application, thereafter providing a basis for assessment of breast tumor angiogenesis by 3D-CEUS and DCE-MRI.

2. Materials and methods

2.1. Study population

From May 2011 to February 2012, 183 consecutive patients with breast lesions treated by a multidisciplinary team approach were entered prospectively into a database in our institution, which was approved by institutional review board committee, and patients' informed consent was obtained. All of our investigation was in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Among these 183 patients, 182 were female, one was male (age range, 20–76 years; mean age, 50.25 years). If a patient had multiple lesions, only the most conspicuous lesion was observed. All patients underwent preoperative baseline US, 2D-CEUS, 3D-CEUS and MRI, and subsequently underwent surgery (modified radical mastectomy or radical mastectomy or conservative breast surgery) within one week. None of them received preoperative neoadjuvant chemotherapy or endocrine therapy. The mean diameter of the lesions was 2.6 cm $\pm$ 1.3 (range, 0.6–6.5 cm).

2.2. Ultrasound examination and evaluation

2.2.1. Ultrasound examination

Conventional US, 2D-CEUS, and 3D-CEUS scanning were performed using the same ultrasound machine Mylab 90 (Esaote, Genoa, Italy). Conventional US and color Doppler US were performed by LA 532 transducer with a frequency of 13–4 MHz, while 2D-CEUS was evaluated by LA 522 transducer with a frequency of 9–3 MHz, the BL 433 volume transducer with a frequency of 15–9 MHz was used for 3D scanning. The contrast agent was SonoVue (BR1, Bracco SpA, Milan, Italy), a sulfur hexafluoride-filled microbubble contrast agent. To avoid interobserver variability, all the US scanning was performed by one radiologist with 5 years of experience in breast CEUS, and one year in breast 3D-CEUS. All ultrasound scanning were performed usually less than one week before surgery.

First, conventional US, including color Doppler US and baseline 3D scanning were carried out, respectively, to observe general features of breast tumors and to select the best tumor imaging in the maximum plane, from which both the tumors and the normal adjacent breast tissue could be observed. In the meantime, the appropriate volume angle was defined so that the whole lesion would be included in the volume data without signal loss. Imaging parameter settings were optimized to ensure high quality images after the target lesion was determined. Subsequently, 2D-CEUS was performed by LA 522 transducer with a frequency of 9–3 MHz. The scanner settings for CEUS were as follows: the selected plane included the lesion and its surrounding normal tissue if possible; range, 70 dB; the image depth was 3 or 4 cm. When the signals from the microbubbles in the large vessels such as axillary vein disappeared, 3D-CEUS was initiated. The contrast agent SonoVue was injected as the same dose and fashion with 2D-CEUS (2.4 mL of SonoVue as a bolus through an antecubital vein, followed by a flush of 5 mL of 0.9% saline). Ten seconds later, 3D-CEUS images were continuously obtained more than five times with the total time over 2 min. The imaging settings for 3D-CEUS was as follows: MI, 0.08–0.13; one focal zone; power output, 3–6%; dynamic range, 40–60 dB; volume angle, 30–50° and the scanning route was consistent with that for 2D-CEUS. During the 3D scanning, the transducer was kept in a stable position without movement and the patient was asked to hold the breath for 5–10 s depending on the size of the volume data and the acquisition mode. Transparent mode and tomographic mode imaging (TMI) were mainly selected to depict neovascularization. After that, the reformatted CEUS images of three orthogonal planes were displayed on the screen. All the data, including CEUS and 3D CEUS images were stored in the hard disk of the ultrasonography machine in the DICOM format for further analysis.

2.2.2. 3D-CEUS imaging analysis

The 3D-CEUS measurements were analyzed by 2 investigators who did not perform US examinations and were blind to surgical, histological information and other imaging findings. After independent interpretations by the two observers, consensus was obtained in conference, and if different assessment were assigned, a consensus reached after discussion. Based on our previously study [21] the characteristics of tumor vasculature were focused to assess as follows: (1) peripheral vessels (presence or absence), their distribution (radial or not), courses (distorted or not) and dilated degree (coarse or not), (2) penetrating vessels (presence or absence) and their courses(running inside tumor or towards center), (3) rim perfusion (presence or absence) and degree (thin/moderate or coarse) (4) intratumoral vessels (presence or absence) and their dilated degree (coarse or not). According to the correlation between the described diagnostic characteristics and the probability of malignancy, a score from 0 to 2 was given, respectively, for each characteristic. The diagnostic and scoring criteria for 3D-CEUS characteristics were summarized in Table 1.

2.3. MRI examination and evaluation

2.3.1. MRI examination

Breast MR imaging was performed on a dedicated breast magnetic resonance imaging (DBMRI) system (Aurora Dedicated Breast MRI Systems, USA) with a dedicated breast coil while the patients