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Research article

Quantitative contrast-enhanced ultrasound evaluation of pathological complete response in patients with locally advanced breast cancer receiving neoadjuvant chemotherapy

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ABSTRACT

Purpose: To clarify whether the quantitative parameters of contrast-enhanced ultrasound (CEUS) can be used to predict pathological complete response (pCR) in patients with locally advanced breast cancer receiving neoadjuvant chemotherapy (NAC).

Material and methods: Fifty-one patients with histologically proved locally advanced breast cancer scheduled for NAC were enrolled. The quantitative data for CEUS and the tumor diameter were collected at baseline and before surgery, and compared with the pathological response. Multiple logistic regression analysis was performed to examine quantitative parameters at CEUS and the tumor diameter to predict the pCR, and receiver operating characteristic (ROC) curve analysis was used as a summary statistic.

Results: Multiple logistic regression analysis revealed that PEAK (the maximum intensity of the time-intensity curve during bolus transit), PEAK%, TTP% (time to peak), and diameter% were significant independent predictors of pCR, and the area under the ROC curve was 0.932(Az_1), and the sensitivity and specificity to predict pCR were 93.7% and 80.0%. The area under the ROC curve for the quantitative parameters was 0.927(Az_2), and the sensitivity and specificity to predict pCR were 81.2% and 94.3%. For diameter%, the area under the ROC curve was 0.786 (Az_3), and the sensitivity and specificity to predict pCR were 93.8% and 54.3%. The values of Az_1 and Az_2 were significantly higher than that of Az_3 ($P = 0.027$ and $P = 0.034$, respectively). However, there was no significant difference between the values of Az_1 and Az_2 ($P = 0.825$).

Conclusion: Quantitative analysis of tumor blood perfusion with CEUS is superior to diameter% to predict pCR, and can be used as a functional technique to evaluate tumor response to NAC.

1. Introduction

Neoadjuvant chemotherapy (NAC) has become a standard treatment for patients with locally advanced breast cancers [1,2]. NAC has the advantage of increasing the rate of breast conservation surgery, and provides an opportunity to monitor an individual patient's response and tailor her therapeutic regimen [3]. Pathological complete response is the most important response indicator for patients with breast cancer receiving chemotherapy. Patients with a pCR have shown improvements in overall survival and long-term disease-free survival compared with patients without a pCR [4]. Recently, pCR rates have increased because of the wide availability of more effective chemotherapy regimens and the use of targeted therapies. Therefore, it is critical to accurately detect whether residual carcinoma is present or absent after NAC.

Morphological response evaluation criteria have been widely used in clinics to evaluate the responses of tumors to chemotherapy. However, there is increasing awareness that anatomical approaches based on measuring the tumor size have substantial limitations. Morphological change in response to chemotherapy often manifests later than changes in underlying metabolic responses [5,6], and in some patients, reduction in size may not occur despite a positive functional response to treatment. Furthermore, some tumors with necrosis and fibrotic scars cannot be differentiated from residual tumor based on morphological imaging alone; therefore, the correlation of the residual tumor size measured by anatomical imaging and pathology after NAC is not good. Besides, newly developed chemotherapies, such as anti-angiogenic drugs, require the assessment of tumor blood flow changes

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instead of tumor morphological changes. Recently, more attention has been paid to functional imaging techniques that are used to depict physiological and cellular processes within tumors, such as the changes in vascularity and metabolism.

With the introduction of second-generation contrast agents, contrast-enhanced ultrasound (CEUS) has created a significant opportunity to visualize the microcirculation of the tumor, and can facilitate continuous and dynamic observation of tumor perfusion [7]. The present study was designed to clarify whether the quantitative parameters of the CEUS imaging could be used to predict pCR in patients with primary breast cancer receiving NAC. In addition, the changes in CEUS quantitative parameters and tumor size after NAC between patients who achieved pCR and those who did not (non-pCR) were compared.

2. Material and methods

2.1. Patients

This retrospective study was approved by the institutional ethics committee. All patients gave written informed consent to undergo the NAC protocol and to participate in the CEUS examination study. From May 2015 to February 2016, 72 female patients with biopsy-proved primary breast cancer undergoing NAC were evaluated using CEUS. The purpose of this study was to correlate the CEUS imaging findings with final pathology; therefore, only patients who underwent a CEUS examination before NAC, after completing NAC infusion, and before surgery were included for analysis. Based on these criteria, 21 patients were excluded: 12 patients did not have a final CEUS examination after NAC, and there were four patients whose specimens were not available at the time of this study. Five patients were excluded because of excessive movement artifacts during CEUS examination.

2.2. Neoadjuvant treatment

All of the patients received four cycles of taxol (80 mg/cm^2) and cisplatin (25 mg/cm^2). Patients with human epidermal growth factor receptor 2 (HER2)/receptor-positive lesions additionally received Herceptin (initial dose, 4 mg per kilogram of body weight; subsequent dose, 2 mg/kg). All patients underwent modified radical mastectomy after the completion of NAC. Surgery was performed within 1 week of the final CEUS examination.

2.3. CEUS examination

Conventional US imaging was performed using MyLab Twice (Esaote, Genoa, Italy) with a 4–13-MHz LA523 linear transducer. The maximum diameter of the tumor was measured at baseline and after NAC using US and correlated with the pathological findings. Color Doppler US was performed to evaluate intralesional vascularity in different planes. Most vascular planes were selected for CEUS. The selected plane included the lesion and its surrounding normal tissue, if possible. CEUS was performed with the aforementioned unit and a 4.5–7.5-MHz LA332 linear transducer.

The contrast agent used in the study was SonoVue (Bracco, Milan, Italy). For contrast-tuned imaging, 2.5 mL of the contrast agent was injected via the antecubital vein in a bolus fashion through a 20-gauge intravenous cannula, followed by a flush with 5 mL of 0.9% normal saline solution. Continuous imaging was performed immediately after injection of the contrast agent and lasted for 6 min. The quantitative acquisition time for time-intensity (T/I) curve analysis was 3 min. All patients underwent pretreatment baseline CEUS examination and a final CEUS examination within a week before surgery.

2.4. Image analysis

Quantitative parameters were obtained using sonographic quantification software (Qontrast, Bracco, Milan, Italy), which was installed in a computer running Windows. A region of interest (ROI) was defined manually around the whole lesion, and Qontrast calculated the average signal intensity of the selected ROI by generating a gamma-varied time-intensity (T/I) curve. Areas of ligaments, fascia, calcifications, and necrosis were avoided. The parameters Peak, TTP (s), RBV, RBF, and MTT (s) (for definitions, see following paragraph) provided by the gamma variate T/I curve were analyzed. The analysis of the quantitative parameters was performed by two radiologists (J.D. and C.F.W.; both of whom have more than 8 years experience in breast CEUS examination). If disagreement occurred, another radiologist (F.H.L., with 11 years experience in breast CEUS examination) reviewed the image until consensus was achieved.

Peak: the maximum intensity of the time-intensity curve during bolus transit ([post-contrast signal – pre-contrast signal]/pre-contrast signal) × 100%.

TTP (s) (time to peak): time needed to reach peak intensity beginning from the time the first microbubble reached the lesion.

RBV (regional blood volume): total blood volume in the ROI region, proportionate to the area under the curve.

RBF (regional blood flow): relative blood flow in the ROI region (RBV/MTT).

MTT (s) (mean transit time): circulation time of contrast agent in the area under investigation.

2.5. Pathological examination and response evaluation

Surgical specimens were cut into 5-mm slices and fixed in 10% neutral-buffered formalin for histological analysis. Pathological diagnosis and the response to NAC were analyzed by two pathologists, who have more than 15 years of experience in breast pathological analysis. If no tumor gross lesion was found in the surgical specimen, the region of the marker left in the breast and the adjacent blocks were examined. Residual diseases after NAC were classified into three groups: (1) no residual cancer was found; (2) no residual invasive cancer was found, but a ductal carcinoma *in situ* was observed; (3) residual invasive cancer was found. In this study, we only evaluated the breast lesions. pCR was defined as a lesion without invasive residual cancer, which included the first two categories [8].

Immunohistochemical staining was performed to identify the following prognostic indicators: estrogen receptor (ER), progesterone receptor (PR), and HER2. The cutoff point for ER and PR positivity was 10%. *HER2* expression was evaluated using fluorescence *in situ* hybridization. Expression of *HER2* was considered positive if the gene-to-chromosome ratio was greater than 2.0.

2.6. Statistical analysis

All statistical evaluations were performed using IBM SPSS Statistics 23 (Armonk, NY, USA). Kolmogorov–Smirnov analysis was performed to assess normal distributions. The Levene test was used to evaluate the homogeneity of variance. The independent samples Student's *t*-test (2-sided, 95% confidence interval) was carried out to assess whether statistically significant changes in the quantitative CEUS parameters correlated with the ultimate treatment response. Logistic regression analysis was used to test the independence of established factors for the prediction of pCR. The diagnostic performance in differentiating between pCR and non-pCR patients was assessed by receiver operating characteristic curves (ROC) and the area under the curves was compared. A *P*-value of 0.05 or less was considered statistically significant. Changes in the quantitative parameters and diameter before and after NAC were calculated using the following formula: ([value before NAC-value after NAC]/value before NAC) × 100%.