Abstract. Neoadjuvant chemotherapy (NACT) is a widely accepted therapeutic option for patients with breast cancer. Although NACT produces good results for breast cancer patients, it has the potential to delay effective treatment in patients with chemotherapy-resistant breast cancer. The purpose of the present study was to evaluate the utility of the pretreatment apparent diffusion coefficient (ADC), which is calculated from diffusion-weighted imaging (DWI), the change in ADC after first administration of NACT, and the change in tumor greatest diameter on ultrasonography in the early prediction of the tumor response to NACT. The response rate of breast tumors to NACT was calculated by the greatest diameter measured by contrast-enhanced MRI obtained before and after NACT. Only the change in ADC was significantly correlated with the response rate. The area under the curve of the change in ADC was sufficiently high (0.90, 95% confidence interval, 0.760-1.040) to discriminate between responders and non-responders. Calculation of the ADC from DWI-MRI was found to be useful for predicting breast tumor response to NACT. Further studies are required to investigate the benefit of changing systemic therapy for breast cancer based on the prediction of the response to NACT by DWI-MRI.

Introduction

Neoadjuvant systemic chemotherapy (NACT) is the accepted approach for women with locally advanced breast cancer and is an option for women with operable breast cancer, particularly when mastectomy rather than conservative surgery is indicated and the patient desires an attempt at breast conservation (1-4). The primary established clinical benefit for NACT compared with postoperative or adjuvant therapy is in the downstaging of large tumors to improve surgical options. NACT has been shown to result in significantly increased rates of breast-conserving surgery without adversely affecting the overall and disease-free survival rates compared to adjuvant chemotherapy (5,6). However, NACT may have potential disadvantages by delaying local therapy in patients whose tumors turn out to be resistant to the treatment. In fact, some breast cancers do not respond to NACT (7,8).

Diffusion-weighted imaging (DWI) was originally implemented to discover acute cerebral infarction, but it has been increasingly used for the evaluation of extracranial sites such as the abdomen, pelvis (9-11) and breast (12-18). The apparent diffusion coefficient (ADC) is calculated from DWI and correlates with water diffusion without any need for injected contrast material. Although it was found that the mean percentage ADC increase was higher in responders than in non-responders after all cycles of NACT in patients with breast cancer (13-17), measuring the response to anticancer agents after final NACT administration is too late. By that time, a large quantity of anticancer-agent has already been administered to the patient, and too much time has passed from the time that the breast cancer was discovered. A way of switching from an ineffective anticancer regimen to another method of treatment at an early point is required. A number of studies have reported that the original ADC value before the start of NACT is useful for predicting tumor response to anticancer agents (15,17,18). After the first and second cycle time points of NACT, an increase in the mean ADC was noted sooner than a reduction in the tumor diameter (12). The purpose of the present study was to compare the usefulness of the pretreatment ADC, the change in ADC after the first cycle of NACT, and the change in tumor greatest diameter measured by gray-scale ultrasonography for the early prediction of tumor response to NACT.

Materials and methods

Patients. Prospective subjects were 24 consecutive female patients with 24 breast cancers diagnosed between March 2009 and October 2010 according to characteristic imaging findings and positive results on core needle biopsy. Each patient was fully informed about the purposes and potential risks and benefits of the study and they provided written, informed consent prior to enrolment. The present study was performed in accordance with the recommendations of the
Declaration of Helsinki. Patient characteristics are shown in Table I. A systemic epirubicin/cyclophosphamide (EC) regimen was administered four times as NACT. The dose with each administration of EC chemotherapy was 90 mg/m² of epirubicin and 600 mg/m² of cyclophosphamide injected every 3 weeks. NACT did not result in adverse events severe enough to warrant withdrawal of therapy after appropriate supportive therapy was provided (such as anti-allergic agents and anti-emetic drugs).

**MRI study (contrast-enhanced MRI).** Dynamic enhanced MRI to measure tumor size was obtained 1-2 days before the first NACT cycle and 10-14 days after the final (fourth) NACT cycle. Dynamic MRI using a three-dimensional fast spoiled gradient-echo sequence (VIBRANT, volume imaging for breast imaging; TR 7.0 ms; TE 4.3 ms; flip angle 10°; FOV 36x36 cm; matrix 512x256; slice thickness 3 mm; gapless; NEX 1) was obtained before and 10 times (every 30 sec) after a bolus injection of 0.1 mmol gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA)/kg by automatic injector at a rate of 3 ml/sec, followed by a 50-ml saline flush. Tumor sizes were measured on delayed enhanced MRI using the image with the maximum tumor diameter and the signal intensity of the tumor relative to the signal intensity of surrounding breast tissue (Figs. 1A,B and 2A,B). Tumor size was calculated as the biaxial diameter product using the maximum and orthogonal diameters on the maximum dimension of each tumor. Tumor response to NACT was calculated as: 100 x [tumor size before NACT] - (tumor size after NACT)]/(tumor size before NACT) according to the Response Evaluation Criteria In Solid Tumors (19).

**MRI study (DWI).** All patients were examined using a 3.0-T MRI unit (Signa EXCITE HDx; GE Healthcare, Milwaukee, WI, USA) with an 8-channel, breast, phased-array coil. DWI was performed 1-2 days before and 5-7 days after the first of four cycles of the NACT regimen. DWI was obtained in 2-3 min periods using a transverse spin-echo echo-planar sequence (repetition time, 4,000 ms; echo time 107.3 ms; matrix size, 128x128; section thickness, 4.5 mm; interslice gapless; four signals acquired; field of view, 400 mm). DWI and ADC maps were acquired using b-values of 0 and 1,500 mm²/s applied in all directions. Quantitative ADC maps were calculated using commercially available software and an imaging workstation (FuncTool and AW 4.3; GE Healthcare). Regions of interest (ROIs) fitted to the lesion shape were placed on breast cancer lesions on the monitor of the FuncTool workstation to calculate ADC (Figs. 1C,D and 2C,D), based on the following formula:

\[
ADC = \frac{1}{n} \frac{\ln(s_0 / s_1)}{(b_1 - b_0)}
\]

where \(\ln\) is the natural log, \(b_0 = 0\) mm²/s, \(b_1 = 1,500\) mm²/s and \(s_0\) and \(s_1\) are the signal intensities of the lesion on images obtained at each b-value. Changes in the ADC value 5-7 days after the first NACT cycle were determined by calculating the percent change in ADC (\%ADC) from baseline (before NACT), with each patient serving as her own control (Figs. 1C,D and 2C,D). The \%ADC from before to after the first NACT regimen was calculated based on the following formula:

\[
\%ADC = 100 \times \frac{(ADC^a - ADC^b)}{ADC^b}
\]

where ADC² is the ADC of the breast cancer before the first NACT regimen and ADC³ is the ADC of the breast cancer after the first NACT regimen.

**Ultrasound study.** Each breast mass was scanned using an ultrasound unit (HI VISION Preirus; Hitachi Aloka Medical, Tokyo, Japan) with a 5- to 13-MHz linear-array transducer. An ultrasound study was performed 1-2 days before and 5-7 days after the first of four cycles of the NACT regimen. Tumor size was measured on the gray-scale ultrasound image (Figs. 1E,F and 2E,F). Tumor response to the first of four cycles of NACT was calculated as \[\%\phi\] (US-1), response rate measured on gray-scale ultrasound image between before and after first-time NACT administration.

**Table I. Clinical manifestations and imaging findings of the cases examined.**

| Variables          | Values          |
|--------------------|-----------------|
| Age (years)        | Mean 54.3       |
| Range              | 32-69           |
| TNM                | I 2             |
|                    | II A 13         |
|                    | II B 6          |
|                    | III A 1         |
|                    | III B 1         |
|                    | III C 0         |
|                    | IV 1            |
| ADC (0) (x10⁻³ mm²/s) | Mean 1.006       |
| Range              | 0.664-1.359     |
| %ADC (%)           | Mean 7.79       |
| Range              | -33.8 - +24.13  |
| ϕ (MRI-0) (mm)     | Mean 29.8       |
| Range              | 13-58           |
| %ϕ (US-1) (%)      | Mean 8.1        |
| Range              | -16.7 - +35.1   |
| Response rate (%)  | Mean 34.1 (14 responders, 10 non-responders) |
| Range              | 0-100           |

TNM, tumor-node-metastasis classification; ADC, apparent diffusion coefficient; ADC (0), ADC value before neoadjuvant chemotherapy (NACT); %ADC, change in ADC value between before and after first-time NACT administration; ϕ (MRI-0), maximum tumor diameter measured on MRI before NACT; %ϕ (US-1), response rate measured on gray-scale ultrasound image between before and after first-time NACT administration.
DWI and tumor size on dynamic MRI and on ultrasound were evaluated by three radiologists, K.K., M.N. and N.H., who were blinded to other clinical information and have >15 years’ experience in breast imaging.

Statistical analysis. Statistical analysis was performed using SPSS version 10.0 software (SPSS Inc., Chicago, IL, USA). Pearson's correlation test was used to measure the linear association between the tumor response rate and the pre-NACT ADC value [ADC(0)], %ADC and pre-NACT maximum tumor diameter measured by MRI [ϕ (MRI-0)] and %ϕ (US-1). Receiver operating characteristic (ROC) analysis was performed to calculate the area under the curve (AUC) to differentiate responders and non-responders by the independent variable with a significant correlation with the dependent variable (response rate) determined by Pearson's correlation test. Two-sided tests were used, with values of p<0.05 indicating statistically significant differences.

Results

Patient characteristics and radiological findings are summarized in Table I. Pearson's correlation test showed a significant correlation between %ADC and the response rate (r=0.597,
p=0.016); none of the other three independent variables were correlated with the response rate (Table II). Therefore, only %ADC was evaluated by ROC analysis (Fig. 3). The AUC of %ADC to differentiate between responders and non-responders on ROC analysis was 0.90 [95% confidence interval, 0.760-1.040]. Breast cancer lesions with high %ADC values responded to NACT (Fig. 1), while those with low %ADC values did not (Fig. 2).

**Discussion**

The early prediction of the effectiveness of NACT has the potential to contribute to breast cancer patient prognosis and cosmetic outcome by facilitating the early alteration of the chemotherapy regimen. Among the independent factors extracted from MRI and ultrasound examinations in the present study, only an early change in the ADC after the first cycle of NACT correlated with the tumor response rate and had a sufficient AUC on ROC analysis to differentiate between responders and non-responders. Measurement of ADC by DWI has been reported to be useful to differentiate lesions and evaluate therapeutic efficacy in the breast and other organs. The ADCs of hepatic benign lesions were significantly greater than those of malignant lesions (9). A significant increase in ADC was observed in metastatic lesions that responded to chemotherapy (10). We previously
predicting the response of breast cancer to NACT (15,17), it was found that the ADC value was the significant parameter in the early prediction of responders and non-responders to anthracycline-based regimen chemotherapy for breast cancer. Further investigations are required to confirm the benefit of early discrimination of responders from non-responders to anthracycline-based regimen. Whether breast cancer determined by DWI-MRI to be unresponsive to an anthracycline-based regimen should be considered (18). Ideally, it should be possible to evaluate the effect of NACT before the start of treatment. An attempt to perform a large-scale study taking into account breast tumor phenotypes was not possible to reproduce this finding in the present study.

Richard et al reported that pretreatment ADC can predict the response of breast cancer to NACT if tumor phenotype is considered (18). Ideally, it should be possible to evaluate the effect of NACT before treatment prior to tumor size measurement (12). In the present study, which included a larger number of patients, it was possible to demonstrate statistically the advantage reported by Pickles et al. Many investigators have verified that both grayscale ultrasound and Doppler sonography have a high ability to differentiate benign and malignant breast lesions (20-22). Ultrasonography has also been reported to be useful in evaluating axillary lymph node metastases, intraductal cancer spread and outcomes of various conservative therapies for breast cancer (23-28). There have also been attempts to use nuclear medical imaging, magnetic spectroscopy, and contrast-enhanced MRI to evaluate glucose metabolism, cell membrane phospholipid metabolism and enhancement features (16,17,29-31). Although DWI-MRI costs more than ultrasonography, it does not require expensive radiological agents or contrast agents. Magnetic spectroscopy is comparatively expensive, requiring a high-sensitivity MRI device, and it must be used in conjunction with contrast-enhanced MRI to detect breast cancer lesions. The fact that the value of DWI-MRI, which provides comparatively greater versatility among the various diagnostic imaging techniques, for predicting early response to NACT has been demonstrated is highly significant. Anthracycline-based regimens, taxane-based regimens, and third-line regimens have been developed as systemic chemotherapy for breast cancer (1-8,28). Human epidermal growth factor receptor 2 (HER2)-directed therapy for HER2-positive breast cancer and endocrine therapy for hormonal receptor-positive breast cancer are other options (28). In the present study, DWI-MRI successfully predicted the early response to an anthracycline-based regimen. Whether breast cancer determined by DWI-MRI to be unresponsive to an anthracycline-based regimen should be treated with a different systemic therapy or by surgical excision is a question for further study.

In conclusion, change in the ADC after the first cycle of NACT correlated well with the tumor response rate of breast cancer. Calculation of ADC by DWI-MRI was useful in discriminating responders from non-responders to anthracycline-based regimen chemotherapy for breast cancer. Further investigations are required to confirm the benefit of early alteration of systemic therapy based on DWI-MRI response prediction.

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