Predicting Pathologic Response to Neoadjuvant Chemotherapy in Breast Cancer by Using MR Imaging and Quantitative ^{1}H MR Spectroscopy[1]

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Purpose: To compare changes in the concentration of choline-containing compounds (tCho) and in tumor size at follow-up after neoadjuvant chemotherapy (NAC) between patients who achieved pathologic complete response (pCR) and those who did not (non-pCR).

Materials and Methods: This study was approved by the institutional review board and was compliant with HIPAA; each patient gave informed consent. Thirty-five patients (mean age, 48 years ± 11 [standard deviation]; range, 29–75 years) with breast cancer were included. Treatment included doxorubicin and cyclophosphamide followed by a taxane-based regimen. Changes in tCho and tumor size in pCR versus non-pCR groups were compared by using the two-way Mann-Whitney nonparametric test. Receiver operating characteristic (ROC) analysis was performed to differentiate between them and the area under the ROC curve (AUC) was compared.

Results: In the pCR group, the tCho level change was greater compared with change in tumor size ($P = .003$ at first follow-up, $P = .01$ at second follow-up), but they were not significantly different in the non-pCR group. Changes in tumor size and tCho level at the first follow-up study were not significantly different between the pCR and non-pCR groups but reached significance at the second follow-up. In ROC analysis, the magnetic resonance (MR) imaging and MR spectroscopic parameters had AUCs of 0.65–0.68 at first follow-up; at second follow-up, AUC for change in tumor size was 0.9, AUC for change in tCho was 0.73.

Conclusion: Patients who show greater reduction in tCho compared with changes in tumor size are more likely to achieve pCR. The change in tumor size halfway through therapy was the most accurate predictor of pCR.

Supplemental material: *http://radiology.rsnajnls.org/cgi/content/full/2512080553/DC1*

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Neoadjuvant chemotherapy (NAC) is the standard treatment for patients with inoperable locally advanced breast cancer. For patients with operable cancer, NAC may be used to shrink the tumor to enable breast-conserving surgery (1–3). Patients who achieve pathologic complete response (pCR) after NAC have favorable disease-free survival rates (4–6). The prediction of pCR from the early response can contribute to a timely adjustment of treatment protocol to help reach this goal, and to avoid unnecessary toxicity of ineffective treatments.

Dynamic contrast material–enhanced magnetic resonance (MR) imaging is increasingly being used to evaluate the response of patients with breast cancer undergoing NAC. There is a strong correlation between the residual lesion size measured at MR imaging and the size determined at pathologic examination after NAC (7–10). However, changes in lesion size on dynamic contrast-enhanced MR images may not be detected until after two cycles of chemotherapy (9). If an early surrogate response indicator could be established to predict final treatment outcome, it would help to achieve the goal of pCR.

While hydrogen 1 (^{1}H) MR spectroscopy has been proved helpful for the diagnosis of breast cancer (11–15), the role of ^{1}H MR spectroscopy for therapy response prediction is less established (14,16,17). One small study showed that changes in signal intensity for the choline-containing compounds (tCho) 24 hours after undergoing NAC correlated with the final tumor size reduction; however, most patients received a single anthracycline-based regimen, which is not the current standard of care (17). The purpose of our study was to compare changes in tCho concentration and in tumor size at follow-up after NAC between patients who achieved pCR and those who did not.

Advances in Knowledge

- In patients with breast cancer, MR spectroscopy performed at 1.5 T shows differences in groups with pathologic complete response (pCR) and incomplete responders (non-pCR) to chemotherapy, with those patients showing greater reduction in choline-containing compounds (tCho) compared with changes in tumor size being more likely to achieve pCR.
- The change in tumor size or tCho at the first follow-up was not significantly different between the pCR and non-pCR groups; at the second follow-up, the changes in both became significant.
- The change in tumor size at the second follow-up was the most accurate predictor of pCR after chemotherapy.

Materials and Methods

Patients

This study protocol was approved by the institutional review board and was compliant with the Health Insurance Portability and Accountability Act. All patients gave written informed consent to undergo the NAC protocol, and a separate consent to participate in the MR imaging/MR spectroscopic study. The eligibility criteria were as follows: patients with histopathologically confirmed breast cancer who underwent NAC from June 2004 to December 2006, those who had undergone baseline MR imaging and ^{1}H MR spectroscopy before treatment and had undergone at least two follow-up imaging studies, and those who underwent surgery after completing NAC. Thirty-seven patients (mean age, 48 years ± 11 [standard deviation], range, 29–75 years) met the criteria to be included in the analysis; two patients were excluded owing to small lesion size (<1.5 cm) because of the difficulty of performing ^{1}H MR spectroscopy to obtain reliable results. The patient characteristics, including age, cancer type, cancer stage, pretreatment tumor size, and receptor status (estrogen, progesterone, and human epidermal growth factor receptor 2 [HER-2]), are listed in Table 1.

Treatment Protocol and MR Imaging Follow-up

All patients received biweekly doses of doxorubicin (Adriamycin; Pharmacia-Upjohn, Kalamazoo, Mich) and cyclophosphamide (AC) as their first-line treatment regimen. After the first two cycles, clinicians evaluated patient response and tolerability and decided to continue with two additional cycles of AC treatment or to switch to a taxane-based regimen (paclitaxel [Taxol; Bristol-Myers Squibb] and carboplatin [Paraplatin; Bristol-Myers Squibb, Princeton, NJ]). For patients who had HER-2–positive tumors, trastuzumab (Herceptin; Genentech, San Francisco, Calif) was given weekly with taxane. Five patients with HER-2–negative tumors also received bevacizumab (Avastin; Genentech, South San Francisco, Calif) biweekly with the taxane regimen. The total treatment period ranged from 105 to 235 days (median, 158 days).

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Abbreviations:
AC = doxorubicin and cyclophosphamide
AUC = area under the ROC curve
HER-2 = human epidermal growth factor receptor 2
K_{ep} = redistribution rate constant
K_{trans} = transfer rate constant
NAC = neoadjuvant chemotherapy
pCR = pathologic complete response
ROC = receiver operating characteristic
tCho = choline-containing compound

Author contributions:
Guarantors of integrity of entire study, H.M.B., J.H.C., R.S.M., O.N., M.Y.S.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; approval of final version of submitted manuscript, all authors; literature research, H.M.B., J.H.C., K.N., H.J.Y., S.B., R.S.M., M.Y.S.; clinical studies, H.M.B., J.H.C., K.N., H.J.Y., S.B., R.S.M., O.N., M.Y.S.; experimental studies, H.M.B., K.N., O.N., M.Y.S.; statistical analysis, H.M.B., J.H.C., K.N., H.J.Y., R.S.M.; and manuscript editing, H.M.B., J.H.C., H.J.Y., S.B., R.S.M., O.N., M.Y.S.

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All patients underwent MR imaging and ^{1}H MR spectroscopy prior to treatment as the baseline. They then underwent at least two follow-up imaging studies. The first follow-up study was performed after 1–2 cycles of AC treatment (median, 20 days) and the second follow-up study was performed after four cycles of AC or two cycles of AC and one of taxane (median, 69 days). One patient did not undergo imaging at the time of first follow-up, and two patients did not undergo imaging at the time of second follow-up. All patients underwent one more follow-up MR imaging study after completing the entire NAC treatment, then underwent definitive surgery after the last MR imaging examination (median, 36 days; range, 4–104 days). The follow-up time and the total treatment period for each patient are also included in Table 1.

Pathologic Examination

Surgical specimens were cut in 5-mm slices and fixed in 10% neutral-buffered formalin to prepare the paraffin blocks. For each block where the gross tumor was evident, several 5-μm slides were cut and stained with hematoxylin-eosin for evaluation. If no gross tumor was found in the surgical specimen, the tissue marker left in the breast was identified and slides from the block containing the marker and the adjacent blocks were examined. Residual disease after NAC was classified in one of three categories: *(a)* no residual malignancy,

Table 1

Clinical Characteristics of 35 Patients

Group and Patient No.	Age (y)	Cancer Type	Initial Tumor Size (cm)	Tumor Stage	Biomarker Status		NAC Duration (d)	First Follow-up (d)	Second Follow-up (d)
					Estrogen/Progesterone Receptor	HER-2			
pCR									
1	33	IDC	2.0	IIB	Negative/negative	Negative	135	9	51
2	52	IDC	2.1	IIB	NA	NA	110	20	69
3	53	IDC	6.8	IIIB	Negative/negative	Positive	139	9	50
4	46	IDC	2.9	IIB	Negative/negative	Negative	138	13	48
5	45	IDC	1.8	IIB	Negative/negative	Positive	138	13	55
6	62	IDC	1.9	IIA	Positive/positive	Negative	153	28	. . .
7	56	IDC	1.6	IIB	NA	Positive	133	8	51
8	40	IDC	6.1	IIIA	Negative/negative	Positive	120	9	71
9	44	IDC	4.5	IIB	Positive/positive	Positive	134	10	37
10	51	IDC	6.9	IIIB	Positive/positive	Positive	153	20	52
11	75	IDC	2.7	IIIB	Negative/negative	Positive	144	23	52
12	40	IDC	3.4	IIB	Negative/negative	Positive	119	20	54
13	32	IDC	2.8	IIB	NA	Negative	132	20	55
14	54	IDC	3.6	IV	Negative/negative	Positive	106	21	49
15	36	IDC	1.5	IIA	NA	Negative	106	22	49
16	31	IDC	8.6	IIIB	Negative/negative	Negative	130	20	47
17	36	IDC	8.0	IV	Negative/negative	Negative	137	20	54
Non-pCR									
18	62	IDC	2.1	IIA	NA	NA	160	. . .	79
19	52	IDC	2.8	IIIC	Positive/positive	Negative	137	15	51
20	56	ILC	2.4	IIA	Positive/positive	Positive	138	13	48
21	43	ILC	1.8	IIB	NA	NA	140	9	64
22	51	IDC	2.7	IIB	Negative/negative	Positive	150	20	69
23	44	IDC	6.5	IV	Positive/positive	Negative	133	12	98
24	29	IDC	8.4	IV	NA	NA	153	13	78
25	41	IDC	3.3	IIB	Negative/negative	Negative	151	6	28
26	47	IDC	4.1	IIIB	Positive/positive	Positive	159	13	99
27	60	IDC	2.2	IIA	Positive/negative	Positive	139	25	57
28	59	IDC	6.1	IIIA	Negative/negative	Negative	133	24	52
29	61	IDC	2.7	IIB	Positive/positive	Negative	142	20	72
30	54	ILC	2.7	IIIB	Negative/negative	Positive	141	21	56
31	51	IDC	3.7	IIIB	Positive/positive	Negative	105	20	48
32	41	IDC	7.31	IIIB	Negative/negative	Negative	152	21	. . .
33	33	IDC	3.4	IIA	Positive/positive	Positive	104	20	49
34	58	IDC	2.8	IIIB	Negative/positive	Positive	147	34	62
35	63	IDC/ILC	2.0	IIB	Negative/negative	Positive	153	29	58

Note.—IDC = invasive ductal carcinoma, ILC = invasive lobular carcinoma, NA = not available.

(b) no residual invasive cancer but ductal carcinoma in situ was present, or *(c)* residual invasive cancer. The most commonly used definition of pCR was no invasive cancer, thus including the first two categories (18).

MR Imaging and ^{1}H MR Spectroscopic Protocol and Data Analysis

The detailed MR imaging, ^{1}H MR spectroscopy, and data analysis procedures (19–26) are included in Appendix E1 (*http://radiology.rsnajnls.org/cgi/content/full/2512080553/DC1*). All patients were examined by using a 1.5-T imager (Eclipse; Philips Medical Systems, Cleveland, Ohio).

Statistical Analysis

The percentage of changes in tumor size and tCho level between first and second follow-up, and also between pCR and non-pCR groups, were compared by using the two-way Mann-Whitney nonparametric test. The significance level was set at $P < .05$. The logistic regression model was built to check the effect of the clinical characteristics listed in Table 1 on the obtained results. The analyses were done *(a)* without adjustment for any clinical variables; *(b)* with adjustment for age, tumor stage, estrogen receptor, progesterone receptor, HER-2, initial tumor size, first follow-up interval, or second follow-up interval; and *(c)* with adjustment for all clinical variables together.

The diagnostic performance in differentiating between pCR and non-pCR patients was assessed by using receiver operating characteristic (ROC) curves. The ROC curves created on the basis of the MR imaging and ^{1}H MR spectroscopic parameters at first and second follow-up were obtained and the area under the ROC curve (AUC) was calculated. In each ROC analysis, the cases with missing data were excluded. Since we focused on analyzing the differentiation power given the changes of parameters, the ROC was only performed for patients whose baseline parameters (size, ^{1}H MR spectroscopy, dynamic contrast-enhanced MR parameters) were available to calculate changes. The nonparametric and ROC analyses were performed by using software (SPSS; SPSS, Chicago, Ill). The post hoc power analysis was also performed to assess the power on the basis of our sample size by using software (PASS; NCSS, Kaysville, Utah).

Results

Tumor size, tCho area arbitrary units, water area arbitrary units, and tCho concentration (millimols per kilogram) of all patients were measured at baseline and first and second follow-up; the last MR imaging studies are listed in Table E1 (*http://radiology.rsnajnls.org/cgi/content/full/2512080553/DC1*).

Pathologic Response

After NAC treatment, definitive surgery revealed pCR in 17 (49%) of 35 patients; 15 patients showed no evidence of malignant cells and two patients showed ductal carcinoma in situ only. The remaining 18 (51%) patients showed residual disease (scattered cells, small foci, or bulk tumor) and despite different residual cancers, they were categorized as one non-pCR group. Most of the non-pCR patients also had substantial reduction in tumor size. In our study, trastuzumab was given to all patients with HER-2–positive tumors and no patients with HER-2–negative tumors, but there was no significant difference in their pCR rates (nine [53%] of 17 patients with HER-2–positive tumors vs seven [50%] of 14 patients with HER-2–negative tumors). Cancer type is a much stronger response predictor (all three invasive lobular carcinoma cases and the mixed invasive ductal/lobular carcinoma case did not achieve pCR).

Lesion Size and tCho Changes in pCR Patients

In the pCR group, 12 (71%) of 17 patients had positive tCho levels before treatment. The change in tCho was significantly higher than the change in tumor size at first ($P = .003$) and second ($P = .01$) follow-up by using the nonparametric test (Fig 1; Table 2). Changes for each patient are shown in Figure 2. Figure 3 demonstrates representative MR imaging and ^{1}H MR spectroscopy from a 40-year-old patient who achieved pCR. In post hoc power analysis for the pCR group, the power to accept the tCho change was larger than change in tumor size for the first (96%) and second (87%) follow-up studies.

Lesion Size and tCho Changes in Non-pCR Patients

In the non-pCR group, 17 (94%) of 18 patients had a positive tCho at baseline. The non-pCR group tCho concentration (median, 2.2 mmol/kg; range, 0–7.0 mmol/kg) was higher compared with that of the pCR group (median, 1.4 mmol/kg; range, 0–5.1 mmol/kg) but did not reach significance ($P = .67$). The change in tCho was not significantly different compared with the change in tumor size at first ($P = .07$) and second ($P = .16$) follow-up by using the non-

Figure 1

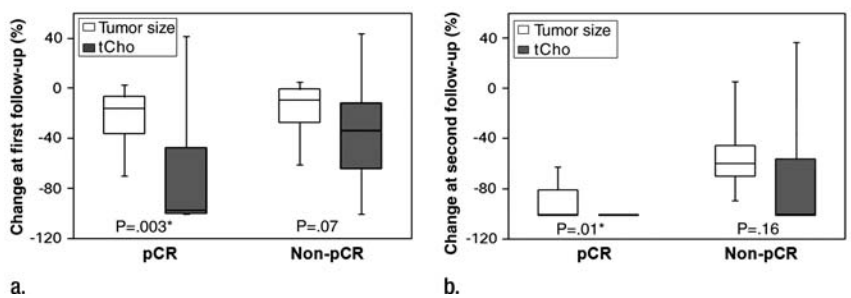

Figure 1: Box plot shows comparison of percentage of changes in tumor size and tCho **(a)** in follow-up study after one to two AC cycles and **(b)** in follow-up study after four AC cycles or two AC cycles followed by one of taxane regimen in pCR versus non-pCR groups. In both follow-up studies, there were significantly higher reductions in tCho level compared with tumor size in pCR group only.

Table 2

Percentage Change in Tumor Size and tCho Concentration at Both Follow-up Examinations

	Tumor Size (cm)			tCho Concentration (mmol/kg)		
MR Study	pCR	Non-pCR	*P* Value	pCR	Non-pCR	*P* Value
Baseline	4.0 (2.9, 1.5–8.6)	3.8 (2.8, 1.8–8.4)	.81	1.4 (0.9, 0–5.1)	2.3 (2.2, 0–7.0)	.67
Change at first follow-up (%)	−22 (−16, 3–70)	−16 (−9, 5 to −60)	.25	−68 (−98, 41 to −100)	−37 (−34, 43 to −100)	.11
Change at second follow-up (%)	−89% (−100%, −62 to −100%)	−51 (−59, −27 to −89)	<.001*	−100 (−100, all −100)	−67 (−100, 36 to −100)	.01*

Note.—Data are the mean value in each group; numbers in parentheses are the median, followed by the range of minimum to maximum.

* Significant ($P < .05$) between pCR and non-pCR group tested by using two-way Mann-Whitney nonparametric test.

parametric test (Fig 1; Table 2). Figure 4 shows the percentage of changes of tCho level and lesion size of patients in the non-pCR group. Two patients had elevated tCho levels at second follow-up and substantial residual tumor (4.6 cm), which was larger compared with the tumor size of the other 33 patients (range, 0–2.8 cm) (Table E1 [*http://radiology.rsnajnls.org/cgi/content/full/2512080553/DC1*]). Figure 5 demonstrates examples of MR imaging and ^{1}H MR spectroscopic images from a 29-year-old patient with invasive residual disease. In post hoc power analysis for the non-pCR group, the power to accept the tCho change was larger than change in tumor size by 70% in the first and second follow-up studies.

Figure 2

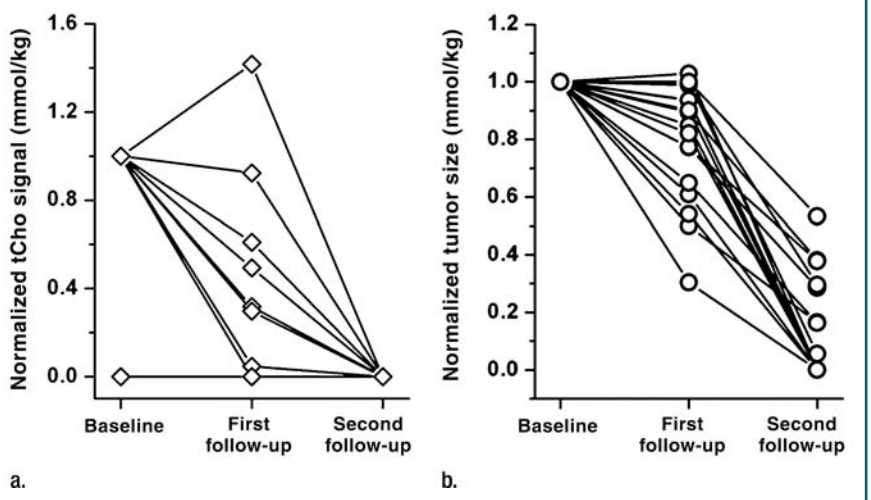

Figure 2: Plots of changes in **(a)** normalized tCho and **(b)** tumor size with treatment in pCR patients. First follow-up study after one to two AC cycles and second follow-up study after four AC cycles or two of AC followed by first cycle of taxane regimen. Five patients did not have detectable tCho at baseline. Of 12 patients who had positive tCho before treatment, seven had detectable tCho at first follow-up. At second follow-up, all patients had substantially smaller tumor sizes and none had detectable tCho.

Comparison of Changes in tCho and Tumor Size between pCR and Non-pCR Groups

Baseline tumor size and tCho were not significantly different between pCR and non-pCR groups (Table 2). The change at first follow-up compared with baseline also did not show significant difference. At second follow-up, both tumor size ($P < .001$) and tCho ($P = .01$) show greater changes in the pCR group than in the non-pCR group. None of the tested parameters (age, tumor stage, tumor size before treatment, biomarker status, and follow-up time after treatment) altered the significant testing results for the changes of both tCho concentration and tumor size at first and second follow-up, indicating that the results were not confounded by different clinical characteristics between the pCR and non-pCR groups. The post hoc power analysis was performed to test the hypothesis that the change in tumor size or tCho concentration during treatment was significantly different between the pCR and non-pCR groups. At first follow-up, the power for change in tumor size was 14% and the power for change in tCho was 41%, which was not sufficient to predict pCR. At second follow-up, the power for change in tumor size was 99% and power for change in tCho was 74%.

Changes of Water Reference Peak at First Follow-up

The internal reference method used the water peak as the reference standard.

To assess the effects of varying water content during chemotherapy, the percentage change of raw data, including the peak area of tCho area arbitrary units measured from the suppressed spectra and the water area arbitrary units measured from the unsuppressed spectra, were calculated (Table E1 [*http://radiology.rsnajnls.org/cgi/content/full/2512080553/DC1*]). All three parameters showed a higher but nonsignificant reduction in the pCR than in the non-pCR group. Although the water peak also showed reduction, it was smaller compared with the change of tCho peak.

Figure 3

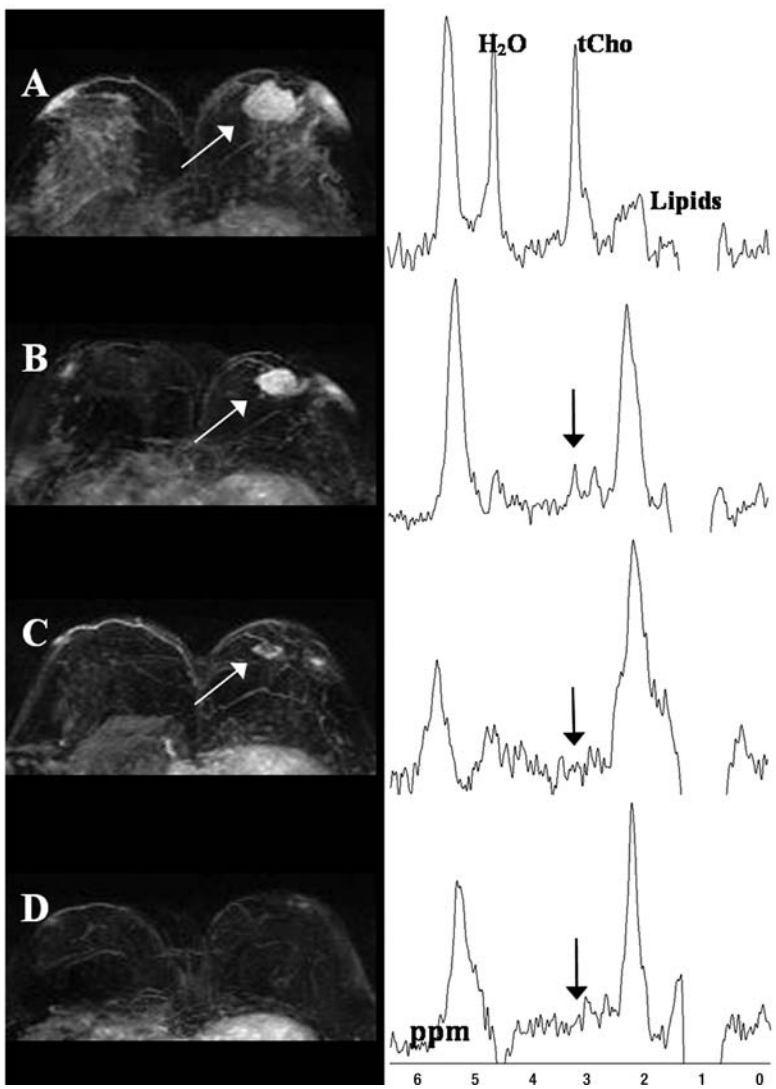

Figure 3: Maximum intensity projection MR images (left) and corresponding MR spectra (right) from 40-year-old patient (patient 12) who achieved pCR. *(A)* Before treatment, 3.4-cm lesion (arrow) shows heterogeneous enhancing pattern. Elevated total tCho peak (right) is visible at 3.23 ppm in water-fat suppressed spectrum. Gaussian model fitting of tCho peak, 2.33 mmol/kg ± 0.54. *(B)* Enhancing lesion (white arrow) and water-fat suppressed spectrum (black arrow) acquired after one AC cycle. Lesion size is reduced to 2.6 cm, tCho peak is visible at 3.22 ppm, and tCho = 1.15 mmol/kg ± 0.25. Change of tCho level shows 51% reduction, tumor size shows 23% reduction. *(C)* At second follow-up after two AC cycles and one of taxane regimen, lesion (white arrow) further shrank to 1.3 cm, indicating good response. *(D)* After all chemotherapy, breast lesion completely regressed. In water-fat suppressed spectrum, tCho peak (arrow) was no longer detectable (100% reduction).

Prediction of pCR, Given Changes in MR Imaging and ^{1}H MR Spectroscopic Parameters, by Using ROC Analysis

For ^{1}H MR spectroscopic parameters, since the ROC was only analyzed on the basis of changes at first and second follow-up, only patients with detectable tCho found at baseline MR imaging were included in the analysis. At first follow-up, the respective AUCs for tumor size, tCho level, and tumor size and tCho level combined were 0.66, 0.67, and 0.72, and were not significantly different between pCR and non-pCR groups. At second follow-up, the respective AUCs were 0.9, 0.73, and 0.92; all showed significant differences ($P < .05$).

The ROC analysis was also performed given the raw ^{1}H MR spectroscopic peak area data. The AUCs were 0.68 for tCho and 0.65 for water peak area at first follow-up and 0.7 for tCho and 0.72 for water peak area at second follow-up. The dynamic contrast-enhanced kinetic parameters (transfer rate constant [K_{trans}] and redistribution rate constant [K_{ep}]) were also analyzed, and the AUC was 0.65 for K_{trans}, 0.66 for K_{ep} at first follow-up, and 0.79 for both at second follow-up. When comparing the differentiation power of all MR imaging or ^{1}H MR spectroscopic parameters, the change in tumor size at second follow-up was the only parameter that achieved a high AUC (0.9).

Discussion

Early change in tumor size measured on MR images is a good predictor of final response after NAC (7–10). However, even if the cells respond to treatment, it takes some time for the tumor to shrink. Substantial research effort has been spent on investigating whether other information provided by MR imaging may serve as earlier response indicators than change in tumor size. These include pharmacokinetic parameters, apparent diffusion coefficient, T2

relaxation time, water-to-fat ratio, and tCho measurement. The results of pharmacokinetic parameters were controversial (27–30). Some researchers found that the pharmacokinetic parameters could predict final response earlier than could size (29), but others could not (27,30). The initial results that used apparent diffusion coefficient as a predictor were encouraging, showing earlier apparent diffusion coefficient change than tumor size reduction (31,32).

These results have to be interpreted within the context of the study, particularly with regard to the drug regimen, and cannot be generalized. Furthermore, most of these studies either used clinical response (later change in tumor size seen at imaging) or pathologic response (between partial responder and nonresponder) as an end point for group comparison. Very few studies attempted to use pCR as an end point, which is the most important response indicator in current oncology. Padhani et al (27) found that change in tumor size after one cycle of NAC can help predict clinicopathologic response after completing all chemotherapy. The pathologic response is used to categorize patients on the basis of their prognosis (eg, pCR patients have favorable prognosis) (4–6,18).

Studies on the use of ^{1}H MR spectroscopy for monitoring the response of patients with breast cancer to NAC have been reported. Kvistad et al (14) and Jagannathan et al (16) demonstrated that qualitative observations of ^{1}H MR spectroscopy at 1.5 T were useful in helping assess the response of locally advanced breast cancer to NAC. Meisamy et al (17) performed quantitative ^{1}H MR spectroscopy at 4.0 T to measure tCho concentration in 13 patients and reported that the change in tCho within 24 hours was significantly different between the clinical responder and nonresponder groups on the basis of later changes in size. Recently, Baek et al (33) reported a similar finding that early tCho change was associated with later clinical response on the basis of reduction in size as seen at MR imaging. These studies used change in size as the end point and suggested that change in tCho may serve as an early indicator for predicting later clinical response. Chemotherapy is known to reduce mitotic count (34) and tumor cellularity (35), which may be the basis for leading to decreased tCho level measured by using ^{1}H MR spectroscopy. Our study is the largest series, to our knowledge, that applies longitudinal quantitative ^{1}H MR spectroscopy to investigate the association of tCho changes during NAC by using the final pathologic response after completing NAC as the end point.

As more therapeutic drugs become available, especially with Food and Drug Administration approval of trastuzumab for HER-2–positive cancer, the pCR rate has been greatly improved (36). The treatment protocol used in our study achieved pCR in 17 (49%) of 35 patients, with most patients in the non-pCR group also having substantial reduction in tumor size. Of these 35 patients, 24 were included in an article by Chen et al (36), which analyzed the correlation between the residual size measured on the last MR image obtained and the pathologic findings of the surgical specimen. For the current study, we focused on the early changes at first and second follow-up, so there was no scientific overlap. The second follow-up study was performed 2 months after start of the treatment course, and the mean reduction in tumor size reached 51% in the non-pCR group and 89% in the pCR group. Therefore, predicting pathologic response in this cohort was potentially more difficult compared with predicting clinical responders versus nonresponders by using less effective treatment protocols.

We found that pCR and non-pCR groups showed different response patterns, particularly in the disparity between changes of tCho and tumor size. In the pCR group, the tCho changes at both first and second follow-up were significantly higher compared with the changes in tumor size, but not in the non-pCR group. Therefore, early reduction in tCho was more pronounced in patients who eventually achieved pCR.

On the other hand, the changes in tumor size and tCho measured in early first follow-up studies could not signifi-

Figure 4

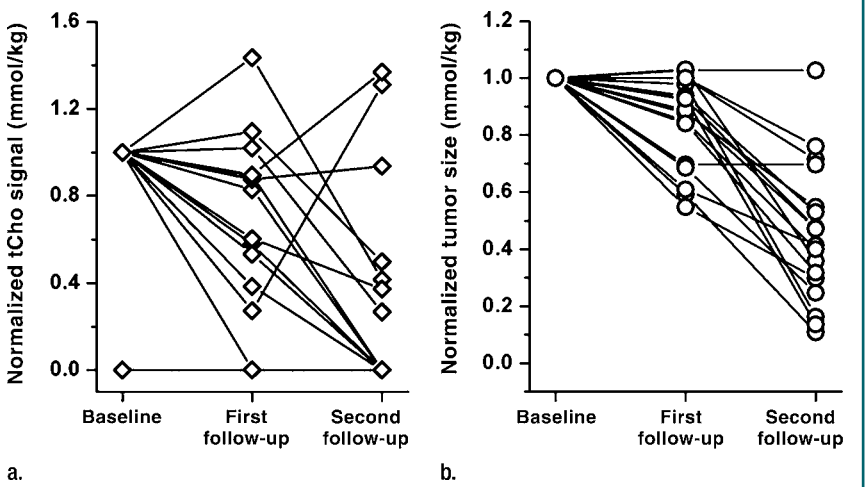

Figure 4: Plots of changes in **(a)** normalized tCho and **(b)** tumor size with treatment in non-pCR patients. One patient did not have detectable tCho at baseline. Of 16 patients who had positive tCho before treatment, 13 had detectable tCho levels at first follow-up. At second follow-up, seven patients had detectable tCho and two had increased tCho (higher than baseline). Patients show wide range of changes in tumor size compared with changes in pCR group in Figure 2.

cantly differentiate between the pCR and non-pCR groups. ROC analysis performed for tumor size, K_{trans}, K_{ep}, and MR spectroscopic parameters (tCho concentration, tCho peak, water peak) were consistent with the nonparametric test results between pCR and non-pCR groups. The AUCs of these parameters were in the range of 0.65–0.68 at first follow-up, not significant between the two groups. At second follow-up, the change in tumor size reached an AUC of 0.9, which was much higher compared with that of 0.79 for K_{trans} and K_{ep}, and 0.70 to 0.73 for the three MR spectroscopic parameters. Again, the results indicated that differentiating between pCR and non-pCR groups was difficult and the change in size at second follow-up was the most accurate predictor.

Figure 5

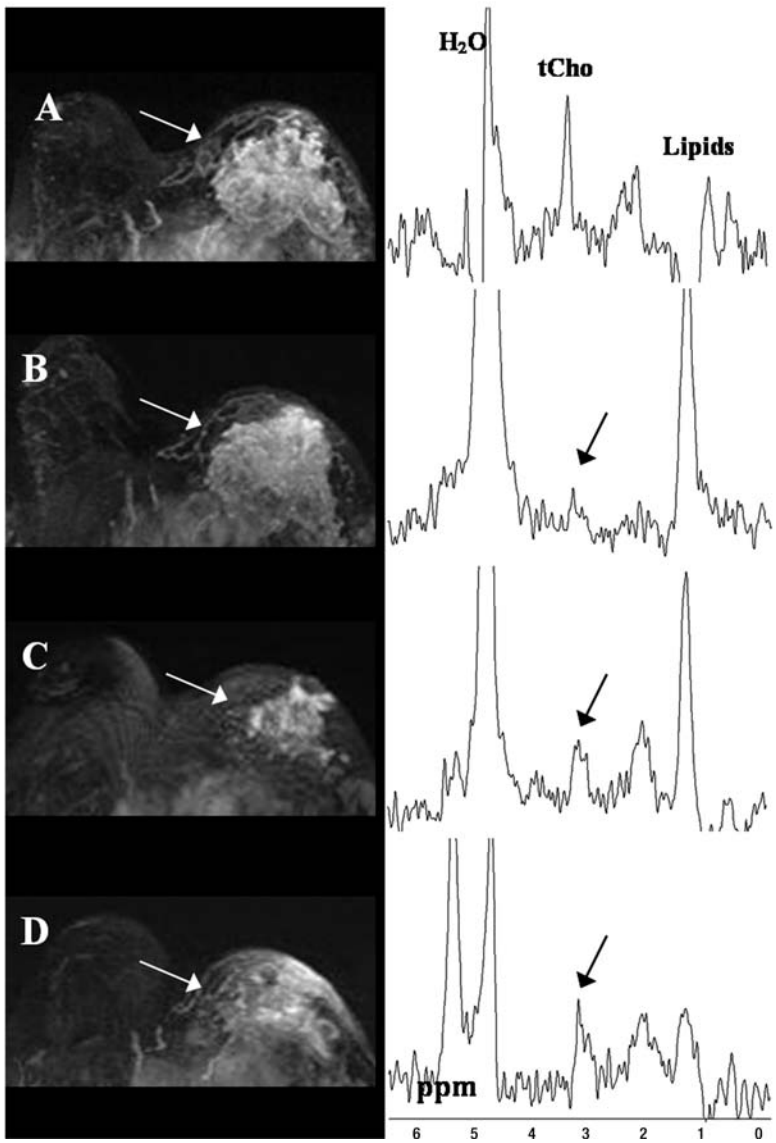

Figure 5: Maximum intensity projection MR images of 29-year-old woman (patient 24) who had residual invasive cancer after completing NAC. *(A)* Before treatment, lesion is 8.4 cm (arrow); tCho peak is visible at 3.22 ppm in water-fat suppressed spectrum (right), and tCho = 0.77 ± 0.11 mmol/kg. *(B)* After one AC cycle, enhanced lesion (white arrow) shrank to 7.4 cm (12% reduction), tCho level (black arrow) decreased to 0.21 mmol/kg (73% reduction). *(C)* At second follow-up after four AC cycles, lesion (white arrow) further shrank to 4.6 cm but tCho concentration increased to 1.01 mmol/kg (black arrow). *(D)* In third follow-up after completing all chemotherapy, tumor increased to 5.5 cm (white arrow). Increase in tCho level (black arrow) at second follow-up seems to predict disease progression at third follow-up.

Other than the difficulty in differentiating between patients who achieved pCR from those in the non-pCR group who had only minimal cancer burden, performing MR spectroscopic quantification over the course of NAC treatment is also challenging. During therapy, as the lesion shrinks, it is more difficult to quantify tCho because there is less tumor tissue to obtain measurements from. To solve this problem, the internal reference method was applied by using the water content as the reference standard. As shown in Table E1 (*http://radiology.rsnajnls.org/cgi/content/full/2512080553/DC1*), there is a substantial change in the water peak of the lesion from baseline to follow-up examinations. Therefore, in addition to tCho concentration, we also performed analyses on the raw data by using the water peak and tCho peak areas. We found that all three parameters showed reduction at first follow-up compared with the baseline, and that all three parameters showed a higher reduction in the pCR than in the non-pCR group. Therefore, the water content indeed changed, but the range was smaller compared with that of tCho.

In addition to water content, T1 and T2 may also vary under normal physiologic conditions (28) but whether it is associated with water content or intrinsic molecular changes with therapy needs to be further investigated (37). Nevertheless, since the result analyzed from the raw tCho peak area was consistent with that of tCho concentration, the effect of changing T1 and T2 during therapy may be small. The various sources that may contribute to the fluctuation are an inherent problem leading to high variation of ^{1}H MR spectroscopic parameters compared with MR imaging.

Our study had limitations. Only one

experienced radiologist obtained size measurements. This was to ensure that consistent criteria could be applied to size measurement. However, whether the same result could be reached by a radiologist with different experience needs to be investigated. Another limitation of our study is the small sample size with different clinical characteristics and treatment protocols. Unfortunately this is a common problem in oncology, because the treatment protocol is often adjusted on the basis of each patient's cancer and response to and tolerability of treatment. Nevertheless, the logistic regression model analysis showed that the clinical characteristic parameters did not affect our results. Whether the lack of effect in the regression analysis is a result of the small sample size warrants further investigation. The treatment protocol used in this study was developed on the basis of the current standard, consisting of anthracycline followed by taxane-based regimens, with the addition of carboplatin. Whether the findings will hold in other less-aggressive regimens remains to be seen.

In conclusion, we found that tCho changes were greater than the changes in tumor size in the pCR group in both first and second follow-up studies but not in the non-pCR group. These results suggest that when the tCho reduction was higher than the reduction in tumor size, the tumor was more likely to achieve pCR. Many patients in the non-pCR group had minimal residual disease, which made differentiation between pCR and non-pCR groups difficult. At second follow-up, the reductions in tCho level and tumor size were significantly higher in the pCR group compared with those in the non-pCR group but not earlier (first follow-up). The AUC was 0.9 given the change in tumor size at second follow-up, which was the highest among all analyzed parameters, suggesting that change in size at second follow-up is the best predictor of pCR.

References

1. Fisher B, Brown A, Mamounas E, et al. Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer: findings from national surgical adjuvant breast and bowel project B-18. J Clin Oncol 1997;15:2483–2493.
2. Fisher B, Bryant J, Wolmark N, et al. Effect of preoperative chemotherapy on outcome of women with operable breast cancer. J Clin Oncol 1998;16:2672–2685.
3. Bonadonna G, Valagussa P, Brambilla C, et al. Primary chemotherapy in operable breast cancer: 8-year experience at the Milan Cancer Institute. J Clin Oncol 1998;16:93–100.
4. Symmans WF, Peintinger F, Hatzis C, et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol 2007;25:4414–4422.
5. Jeruss JS, Mittendorf EA, Tucker SL, et al. Combined use of clinical and pathologic staging variables to define outcomes for breast cancer patients treated with neoadjuvant therapy. J Clin Oncol 2008;26:246–252.
6. Rastogi P, Anderson SJ, Bear HD, et al. Preoperative chemotherapy: updates of National Surgical Adjuvant Breast and Bowel Project protocols B-18 and B-27. J Clin Oncol 2008;26:778–785.
7. Esserman L, Kaplan E, Partridge S, et al. MRI phenotype is associated with response to doxorubicin and cyclophosphamide neoadjuvant chemotherapy in stage III breast cancer. Ann Surg Oncol 2001;8:549–559.
8. Balu-Maestro C, Chapellier C, Bluese A, Chanalet I, Chauvel C, Largillier R. Imaging in evaluation of response to neoadjuvant breast cancer treatment benefits of MRI. Breast Cancer Res Treat 2002;72:145–152.
9. Rieber A, Brambs HJ, Gabelmann A, Heilmann V, Kreienberg R, Kuhn T. Breast MRI for monitoring response of primary breast cancer to neoadjuvant chemotherapy. Eur Radiol 2002;12:1711–1719.
10. Choyke PL, Dwyer AJ, Knopp MV. Functional tumor imaging with dynamic contrast enhanced magnetic resonance imaging. J Magn Reson Imaging 2003;17:509–520.
11. Mackinnon WB, Barry PA, Malycha PL, et al. Fine-needle biopsy specimens of benign breast lesions distinguished from invasive cancer ex vivo with proton MR spectroscopy. Radiology 1997;204:661–666.
12. Gribbestad IS, Sitter B, Lundgren S, Krane J, Axelson D. Metabolite composition in breast tumors examined by proton nuclear magnetic resonance spectroscopy. Anticancer Res 1999;19:1737–1746.
13. Roebuck JR, Cecil KM, Schnall MD, Lenkinski RE. Human breast lesions: characterization with proton MR spectroscopy. Radiology 1998;209:269–275.
14. Kvistad KA, Bakken IJ, Gribbestad IS, et al. Characterization of neoplastic and normal human breast tissues with in vivo 1H MR spectroscopy. J Magn Reson Imaging 1999;10:159–164.
15. Katz-Brull R, Lavin PT, Lenkinski R. Clinical utility of proton magnetic resonance spectroscopy in characterizing breast lesions. J Natl Cancer Inst 2002;94:1197–1203.
16. Jagannathan NR, Kumar M, Seenu V, et al. Evaluation of total choline from in-vivo volume localized proton MR spectroscopy and its response to neoadjuvant chemotherapy in locally advanced breast cancer. Br J Cancer 2001;84:1016–1022.
17. Meisamy S, Bolan PJ, Baker EH, et al. Neoadjuvant chemotherapy of locally advanced breast cancer: predicting response with in vivo 1H MR spectroscopy—a pilot study at 4 T. Radiology 2004;233:424–431.
18. Mazouni C, Peintinger F, Wan-Kau S, et al. Residual ductal carcinoma in situ in patients with complete eradication of invasive breast cancer after neoadjuvant chemotherapy does not adversely affect patient outcome. J Clin Oncol 2007;25:2650–2655.
19. Bottomley PA. Spatial localization in NMR spectroscopy in vivo. Ann N Y Acad Sci 1987;508:333–348.
20. Ordidge RJ, Bendall MR, Gordon RE, Connelly A. Volume selection for in vivo spectroscopy. In: Govil G, Khetrapal CL, Sarans A, eds. Magnetic resonance in biology and medicine. New Delhi, India: Tata-McGraw-Hill, 1985; 387–397.
21. Haase A, Frahm J, Hänicke W, Matthaei D. 1H NMR chemical shift selective (CHESS) imaging. Phys Med Biol 1985;30:341–344.
22. Doddrell DM, Galloway G, Brooks W, et al. Water signal elimination in vivo, using suppression by mistimed echo and repetitive gradient episodes. J Magn Reson 1986;70:176–180.
23. Naressi A, Couturier C, Devos JM, et al. Java-based graphical user interface for the MRUI quantitation package. MAGMA 2001;12:141–152.
24. Knijn A, de Beer R, van Ormondt D. Frequency-selective quantification in the time domain. J Magn Reson 1992;97:444–450.
25. van den Bos A. Parameter estimation. In: Sydenham PH, ed. Handbook of measurement science. Chichester, England: Wiley, 1982.
26. Baik HM, Su MY, Yu H, Mehta R, Nalcioglu O. Quantification of choline-containing com-

pounds in malignant breast tumors by 1H MR spectroscopy using water as an internal reference at 1.5 T. MAGMA 2006;19:96–104.

27. Padhani AR, Hayes C, Assersohn L, et al. Prediction of clinicopathologic response of breast cancer to primary chemotherapy at contrast-enhanced MR imaging: initial clinical results. Radiology 2006;239:361–374.

28. Manton DJ, Chaturvedi A, Hubbard A, et al. Neoadjuvant chemotherapy in breast cancer: early response prediction with quantitative MR imaging and spectroscopy. Br J Cancer 2006;94:427–435.

29. Pickles MD, Lowry M, Manton DJ, Gibbs P, Turnbull LW. Role of dynamic contrast enhanced MRI in monitoring early response of locally advanced breast cancer to neoadjuvant chemotherapy. Breast Cancer Res Treat 2005; 91:1–10.

30. Yu HJ, Chen JH, Mehta RS, Nalcioglu O, Su MY. MRI measurements of tumor size and pharmacokinetic parameters as early predictors of response in breast cancer patients undergoing neoadjuvant anthracycline chemotherapy. J Magn Reson Imaging 2007;26: 615–623.

31. Lee KC, Moffat BA, Schott AF, et al. Prospective early response imaging biomarker for neoadjuvant breast cancer chemotherapy. Clin Cancer Res 2007;13(2 pt 1):443–450.

32. Pickles MD, Gibbs P, Lowry M, Turnbull LW. Diffusion changes precede size reduction in neoadjuvant treatment of breast cancer. Magn Reson Imaging 2006;24:843–847.

33. Baek HM, Chen JH, Nalcioglu O, Su MY. Proton MR spectroscopy for monitoring early treatment response of breast cancer to neoadjuvant chemotherapy. Ann Oncol 2008;19:1022–1024.

34. Adams AL, Eltoum I, Krontiras H, Wang W, Chhieng DC. The effect of neoadjuvant chemotherapy on histologic grade, hormone receptor status, and HER2/neu status in breast carcinoma. Breast J 2008;14:141–146.

35. Rajan R, Poniecka A, Smith TL, et al. Change in tumor cellularity of breast carcinoma after neoadjuvant chemotherapy as a variable in the pathologic assessment of response. Cancer 2004;100:1365–1373.

36. Chen JH, Feig B, Agrawal G, et al. MRI evaluation of pathologically complete response and residual tumors in breast cancer after neoadjuvant chemotherapy. Cancer 2008; 112:17–26.

37. Tan PC, Pickles MD, Lowry M, Manton DJ, Turnbull LW. Lesion T2 relaxation times and volumes predict the response of malignant breast lesions to neoadjuvant chemotherapy. Magn Reson Imaging 2008;26:26–34.

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