TRIPLE-NEGATIVE BREAST CANCER (TNBC) ACCOUNTS FOR ABOUT 10%–20% OF ALL BREAST CANCERS, AND IT IS NEGATIVE FOR ESTROGEN RECEPTOR, PROGESTERONE RECEPTOR, AND HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2 (1). COMPARED WITH OTHER TYPES OF BREAST CANCER, TNBC IS CONSIDERED MORE AGGRESSIVE, WITH A HIGHER RECURRENCE RATE AND DECREASED OVERALL SURVIVAL (1,2). WITHIN THE TNBC GROUP, PATHOLOGIC COMPLETE RESPONSE (pCR) TO NEOADJUVANT SYSTEMIC THERAPY (NAST) IS STRONGLY CORRELATED WITH IMPROVED DISEASE-FREE SURVIVAL AND OVERALL SURVIVAL (3). BECAUSE ONLY ABOUT 20%–50% OF PARTICIPANTS WITH TNBC WILL ACHIEVE pCR, EARLY ASSESSMENT OF THE TREATMENT RESPONSE IS BENEFICIAL (4–6). FOR EXAMPLE, PARTICIPANTS WITH PREDICTED NON-pCR MAY BE DIRECTED TOWARD MORE AGGRESSIVE OR POTENTIALLY MORE EFFECTIVE NOVEL THERAPIES AT AN EARLY STAGE.

THE EFFECTIVENESS OF NAST IS MOST COMMONLY ASSESSED BY THE CHANGE IN TUMOR SIZE BASED ON CONVENTIONAL BREAST IMAGING (EG, MAMMOGRAPHY, US) AND CLINICAL EXAMINATION (7). THE APPROPRIATENESS CRITERIA OF THE AMERICAN NATIONAL INSTITUTES OF HEALTH/ATIONAL CANCER INSTITUTE (GRANTS R01 CA231513 AND P30 CA016672) AND THE CANCER PREVENTION AND RESEARCH INSTITUTE OF TEXAS MULTI-INVESTIGATOR RESEARCH AWARD (RP160710-C1-CPRIT).

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Conflicts of interest are listed at the end of this article.

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Neoadjuvant Systemic Therapy in TNBC Using APT-weighted CEST MRI

Abbreviations

APTw = amide proton transfer–weighted, B1rms = root-mean-square value of the MRI effective component of the radiofrequency magnetic field, C2+ = after two cycles of NAST, C4+ = after four cycles of NAST, CEST = chemical exchange saturation transfer, Lor3.5 = Lorentzian line shape fitting at 3.5 ppm, MTRasym = magnetic transfer ratio asymmetry, NAST = neoadjuvant systemic therapy, pCR = pathologic complete response, ROI = region of interest, TNBC = triple-negative breast cancer

Summary

In a study of participants with triple-negative breast cancer undergoing neoadjuvant systemic therapy, amide proton transfer–weighted chemical exchange saturation transfer MRI signal significantly decreased after two therapy cycles in participants with pathologic complete response but not in those with nonpathologic complete response.

Key Points

- Quantitative measurements from chemical exchange saturation transfer (CEST) MRI should be carefully performed to ensure that the acquisition and analysis methods are appropriately combined.
- A decrease in amide proton transfer–weighted (APTw) CEST signal was observed after neoadjuvant systemic therapy was administered to participants with triple-negative breast cancer.
- The decrease in APTw CEST signal showed a significant early response in participants with pathologic complete response (pCR) after two therapy cycles but was unable to differentiate participants with pCR from participants with non-pCR.

Keywords

Molecular Imaging-Cancer, Molecular Imaging-Clinical Translation, MR-Imaging, Breast, Technical Aspects, Tumor Response, Technology Assessment

College of Radiology recommend the use of MRI in addition to mammography and US in evaluating response of breast cancer to NAST; as a result, at many radiology centers MRI is performed at baseline and upon completion of treatment for improved assessment of treatment response (8). Morphologic changes often occur later during treatment, however (9,10). There are no established guidelines for assessment of early treatment response to NAST in those with breast cancer. Therefore, noninvasive methods that can assess the response to NAST early in treatment are highly desired.

Chemical exchange saturation transfer (CEST) MRI is a molecular imaging method that probes the saturation transfer between protons on water and endogenous biomolecules or exogenous agents (Fig 1) (11). Amide proton transfer–weighted (APTw) CEST MRI generates CEST image signal via the amide protons located on the backbone of mobile proteins and peptides, which resonate at an average of approximately 3.5 ppm downfield from the water protons (12,13). Tumor cells have an increased concentration of cytosolic mobile proteins owing to increased metabolic activities and cellular density, which may be monitored with the APTw CEST MRI signal intensity from amide protons (14,15). Notably, CEST signal can also be generated through radiofrequency saturation of exchangeable protons in metabolites, which are often upregulated in aggressive tumors (16). Because selective saturation at or around 3.5 ppm does not necessarily exclude the detection of these metabolites, CEST at 3.5 ppm is typically described as weighted toward APT from mobile proteins rather than representing only mobile proteins. APTw CEST MRI has been investigated for tumor diagnosis as well as treatment assessment, primarily for brain cancer studies (13,17). APTw CEST MRI has also been developed for breast cancer studies (17–25), including a few studies that have investigated breast cancer treatment response to NAST (18,24,25). These studies have typically evaluated a variety of breast cancer types in the same study, have tested saturation powers ranging from 0.5 to 2.0 µT, and have used different analysis methods. Owing in part to the variety of experimental designs, these studies of breast cancer have not reached a consensus regarding the value of APTw CEST MRI for breast cancer imaging.

The in vivo APTw CEST signal is usually small, which can be challenging to quantitatively measure. A higher saturation power can increase CEST signal and improve quantitative measurements from APTw CEST MRI. However, a higher saturation power tends to broaden a CEST spectrum (a plot of percent water signal vs saturation frequency, also known as a z-spectrum) (26), and broader spectral features can be more challenging to analyze. A variety of analysis methods have been developed to evaluate these CEST spectra features, including magnetic transfer ratio asymmetry (MTRasym), which evaluates the asymmetry in a CEST spectrum (27,28), and Lorentzian line shape fitting, which evaluates the shape of a portion of the CEST spectrum (29).

The first aim of our study was to compare two saturation power levels (0.9 and 2.0 µT) and two analysis methods (MTRasym and Lorentzian line shape fitting at 3.5 ppm [Lor3.5]) to determine optimal combinations of APTw CEST acquisition and analysis methods for evaluating treatment response. Our second aim was to investigate if APTw CEST MRI could be used to predict pCR versus non-pCR in participants with TNBC receiving NAST.

Materials and Methods

This prospective study used the CEST imaging sequence that is provided by GE Healthcare to all users of their instruments. The authors who were not employees of or consultants for GE Healthcare had control of all data and information that might present a conflict of interest.

Participant Sample

Our study was approved by our local institutional review board and was performed in full accordance with the Health Insurance Portability and Accountability Act. Written informed consent was obtained from each participant enrolled in the study. A total of 51 participants with stage I–III TNBC were enrolled in our prospective clinical trial (DCE-MRI and MBI in Assessing Tumor Response to Chemotherapy in Patients With Triple Negative Breast Cancer [NCT02744053]) at our institution from January 31, 2019, through November 11, 2019. These participants met the inclusion criteria of having a primary tumor measuring 1.0–5.0 cm in diameter as diagnosed by anatomic MRI; an estimated glomerular filtration rate of greater than or equal to 60 mL/min and without a history of renal disease; age 18 years or older; not pregnant; and a body mass in-
at all three time points. The C2 time point was optional per protocol. The missed scans at C4 occurred primarily because of participant discomfort caused by the disease and/or treatment. Pathology reports following surgery were available for 26 of the 30 participants. Of these 26 participants, 15 had pCR, and 11 had non-pCR. pCR was defined as no residual invasive disease in the breast or resected nodal tissue. The results from these 26 participants who had known outcomes and had undergone CEST MRI at all time points were used to evaluate whether APT W CEST MRI can evaluate the early response to NAST to predict pCR or non-pCR.

**MR Image Acquisition**

The participants were scanned using a Discovery MR750 or MR750w whole-body MRI scanner (GE Healthcare) using an eight-channel bilateral breast coil, with the participant in a prone position. Because the participants were scanned in a prone position, breathing motion was minimized, and no active or postprocessing motion correction methods were adopted in this study. Our imaging protocol included T1-weighted and T2-weighted anatomic scans, diffusion-weighted MRI, APT W CEST MRI, and dynamic contrast-enhanced MRI. CEST MRI was performed before dynamic contrast-enhanced MRI to avoid the potential effects of the contrast agent on CEST signal.

CEST MR images were obtained using a field of view–optimized and constrained undistorted single-shot fast spin-echo sequence. The images were obtained with echo time of 33.6 msec, repetition time of 6 seconds, centric view ordering, section thickness of 5 mm, field of view of 160 mm × 128 mm, pixel size of 1.2 × 1.2 mm, and single image section in an axial orientation. The single imaging section included the tumor at its largest diameter in the axial view that also excluded the effect from the biopsy mark clip. If a participant responded well to NAST and had a minimal residual tumor volume, the placement of the imaging section was guided by the biopsy clip.

We used fat saturation pulses immediately before the image acquisition sequence because the presence of fat signals is known to reduce amide proton transfer–type MTR asym measurements (20,22,23,30). Our acquisition method was used to image only a single side of the breast with a small field of view, resulting in a more homogeneous B₀ field that was generally within ±0.5 ppm, thereby ensuring more effective fat suppression.

The CEST saturation period was achieved using phase-cycled radiofrequency pulses (31) with a root-mean-square value of the MRI effective component of the radiofrequency magnetic field (B_1rms) of 0.9 and 2.0 mT. The phase-cycled radiofrequency saturation consisted of a train of rectangular pulses with a width of 0.232 msec and an interpulse delay of 0.328 msec. The duty cycle was 41%. The total saturation time was 3500 msec for 0.9-mT saturation and 2000 msec for 2.0-mT saturation, which maintained a specific absorption rate below the maximum safety limit. We acquired 29 equally spaced saturation frequencies in the CEST spectrum from −7 to 7 ppm in 0.5-ppm increments. A reference image was obtained without CEST saturation.
The water saturation shift referencing method was used to correct for the field inhomogeneity, which was acquired with the CEST scans and used the same acquisition method but with a $B_1^{+\text{iso}}$ of 0.42 $\mu$T and with 11 equally spaced saturation frequencies from −1.88 to 1.88 ppm in 0.418-ppm increments (32). The total scan time was 4 minutes 18 seconds, including the water saturation shift referencing method for each CEST sequence.

Data Analyses

All data analyses were performed in a blinded fashion, without knowledge of pCR or non-pCR status until the end of the study. The tumor regions of interest (ROIs) were drawn manually on the CEST images by three experienced breast radiologists with 15, 7, and 7 years of experience (M.B., A.H.A., and R.M.M.M., respectively) under the guidance of the dynamic contrast-enhanced MR images. The biopsy marker clip and necrosis were excluded from the ROI. In cases of complete imaging response without a visible residual tumor, the ROI was drawn on the tumor bed. To account for possible movements between the two CEST scans with different saturation power levels, manual adjustments of the tumor ROI were made as needed after the ROI was copied from one CEST scan to the other. The longest diameter of the tumor was also measured for all available time point on the basis of the delayed phase of the dynamic contrast-enhanced MR images.

The $B_1$-corrected images were automatically generated with the MRI instrument. MTR asym analysis (27,28) and Lorentzian line shape fitting (29) were performed for APTw CEST MRI analysis on a pixel-by-pixel basis using custom routines developed in MATLAB (MathWorks), for a total of four combinations of acquisition and analysis methods. MTR asym analysis is defined as follows:

$$\text{MTR}_{\text{asym}}(\Delta \omega) = \frac{S_{\text{sat}}(\Delta \omega) - S_{\text{sat}}(0)}{S_0} \quad (1),$$

where $\Delta \omega$ is the saturation frequency, $S_{\text{sat}}(\Delta \omega)$ is the signal acquired at $\Delta \omega$, and $S_0$ is the signal acquired without saturation. A z-spectrum is a plot of percent water signal ($S_{\text{sat}}/S_0$) versus $\Delta \omega$ (Fig 1). MTR asym averaged between 3.0 and 4.0 ppm was used to improve the signal-to-noise ratio. A model (Eqq (2,3)) consisting of two Lorentzian lines—the water line at 0 ppm and the amide line at 3.5 ppm—was used to fit the z-spectrum on a pixel-by-pixel basis.

$$Z(\omega) = c - \sum_{j=1}^{2} L_j(\omega) \quad (2),$$

with

$$L_j(\omega) = a_j \frac{1}{2\pi} \frac{\Gamma_j}{(\omega - \omega_j)^2 + \Gamma_j^2} \quad (3),$$

where $c$ is a baseline term to account for the magnetization transfer effects, $a_j$ is the height parameter of the Lorentzian lines, $\Gamma_j$ is the full width at half maximum of the Lorentzian lines, $\omega$ is the experimental saturation frequency, $i = 1,2$ represents water and amide pools, respectively, and $\omega_0$ is 0 ppm and 3.5 ppm for the water and amide pools, respectively. To minimize the influence of the nuclear Overhauser enhancement effects on the Lorentzian line shape fitting, only the points from 7 to −1 ppm in the z-spectrum were used. The magnitude of the fitted Lorentzian line shape at 3.5 ppm ($\Delta m_{3.5}$ or Lor3.5) was used to characterize the APTw CEST effect.

Statistical Analysis

We determined the group average APTw CEST signals at baseline, C2, and C4 time points for each of the four combinations of CEST MRI scan acquisition and analysis methods. The CEST signals were first compared between the three time points (without using pathologic response information) using unpaired (51 participants) and paired (30 participants) Kruskal-Wallis tests for the four acquisition and analysis combinations. Then the CEST signals of the 26 participants with known pCR and non-pCR status from pathology reports were evaluated using the Friedman test to determine if APTw CEST MRI could be used to differentiate pCR from non-pCR participants. Separately, the change in the APTw CEST signals, C2–baseline, C4–baseline, and C4–C2, was determined for each participant and evaluated using the Mann-Whitney test for both groups.

We also evaluated the changes in tumor diameter during NAST for 24 participants who had the longest tumor diameters measured for all time points and who had known pCR and non-pCR status. The group average longest tumor diameters were compared between pCR and non-pCR groups at baseline, C2, and C4 using the Mann-Whitney test. Finally, the changes in the longest tumor diameters, C2–baseline and C4–baseline, were compared between pCR and non-pCR groups using the Mann-Whitney test. All statistical analyses were performed using GraphPad Prism (version 8; GraphPad Software). $P$ value less than .05 was considered statistically significant.

Results

Participant Overview

A total of 51 participants with stage I–III TNBC participated in our study. These participants had a mean age of 51 years (range, 26–79 years), a mean longest tumor diameter of 3.5 cm (range, 1.5–8.7 cm), and invasive ductal, invasive lobular, and/or metaplastic lesions as confirmed by histopathologic evaluation (Table). The mean number of days between baseline and C2 was 34 days (range, 24–61 days), between C2 and C4 was 31 days (range, 22–84 days), and between baseline and C4 was 64 days (range, 50–145 days).

MTR asym Analysis Method with 0.9- and 2.0-$\mu$T Saturation Power

The MTR asym maps and their corresponding ROI-averaged z-spectra and MTR asym spectra are shown for representative participants with pCR and non-pCR from baseline to C4 scans in Figure 2. The z-spectra obtained with 2.0-$\mu$T saturation were compared between pCR and non-pCR from baseline to C4 scans in Figure 2.
**Participant Characteristics**

| Characteristic                  | Acquisition and Analysis Optimization | Treatment Response Differentiation |
|---------------------------------|---------------------------------------|-----------------------------------|
| No. of participants            | 51*                                   | 26†                               |
| Age (y)                         | 51 (26–79)                            | 50 (32–78)                        |
| Stage at diagnosis (T)          |                                       |                                   |
| T1                              | 12 (24)                               | 5 (19)                            |
| T2                              | 32 (63)                               | 20 (77)                           |
| T3                              | 7 (14)‡                               | 1 (4)                             |
| Histopathologic finding         |                                       |                                   |
| Invasive ductal                 | 46 (90)                               | 24 (92)                           |
| Invasive lobular                | 1 (2)                                 | 0 (0)                             |
| Metaplastic                     | 4 (8)                                 | 2 (8)                             |
| Pathologic response             |                                       |                                   |
| pCR                             | 15 (58)                               |                                   |
| Non-pCR                         | 11 (42)                               |                                   |

Note.—Age shown as mean with range in parentheses, all other values shown as numbers with percentages in parentheses. T1 = tumor ≤ 20 mm at its widest area, T2 = tumor is > 20 mm and ≤ 50 mm, T3 = tumor is > 50 mm. pCR = pathologic complete response.

* Participant group used to determine the optimal combination of amide proton transfer–weighted chemical exchange saturation transfer (APTW CEST) MRI acquisition and analysis methods.
† Participant group used to determine if APTW CEST MRI can differentiate pCR from non-pCR.
‡ Percentage total not equal to 100 owing to rounding.

**Figure 2:** The magnetic transfer ratio asymmetry (MTR asym) maps averaged between 3.0 and 4.0 ppm using (A, B) 2.0-μT saturation power and (C, D) 0.9-μT saturation power at baseline, after two cycles (C2), and after four cycles (C4) of neoadjuvant systemic therapy in a 41-year-old woman with triple-negative breast cancer with pathologic complete response (pCR) and a 60-year-old woman with non-pCR. The region of interest—averaged z-spectra (black circles connected with a black line, a plot of percent water signal against saturation frequency) and the MTR asym spectra (blue) of (A) and (C) are shown in (B) and (D), respectively. The images were acquired in the axial plane without contrast agent using single-shot fast spin-echo sequence. These results show that 2.0 μT should be used when evaluating MTR asym. BL = baseline.
saturation power were relatively smooth, and no obvious fat residual signals or nuclear Overhauser enhancement effects were observed (black spectra in Fig 2B). The 0.9-μT z-spectra were noisier than the 2.0-μT spectra. The MTR asym analysis using 2.0-μT saturation power clearly showed APTw CEST between 3 and 4 ppm, while APTw CEST between 3 and 4 ppm was less evident at 0.9 μT (blue spectra in Fig 2). MTR asym was higher at 2.0 μT than at 0.9 μT at baseline, C2, and C4 time points.

**Lorentzian Analysis with 0.9-μT and 2.0-μT Saturation Power**

The Lor3.5 maps and their corresponding ROI-averaged fitted lines for the same two participants are shown in Figure 3. The z-spectra obtained with 0.9-μT saturation power had spectral features that were narrower than z-spectra at 2.0 μT (red spectra in Fig 3). These narrower features allowed APTw CEST at 3.5 ppm to be more evident. The Lor3.5 values at 0.9 μT were higher at 2.0 μT than at 0.9 μT at baseline, C2, and C4 time points.

**Comparison of Analysis Methods and Saturation Power**

We evaluated the changes in APTw CEST from baseline to C2 to C4 for the group average MTR asym and Lor3.5 acquired using both saturation power levels for 51 participants using an unpaired analysis (for all participants regardless of missing scans at some time points) and for 30 participants using a paired analysis (for participants who underwent scanning at all three time points) (Fig 4, Table E1 [supplement]). The Lor3.5 at baseline was higher at 0.9 μT than at 2.0 μT (Fig 4A, 4B). Both the unpaired and paired Lor3.5 values at 0.9 μT showed a significant signal decrease at C2 and C4 compared with baseline (P < .01). The Lor3.5 values at 2.0 μT did not show a statistically significant change between scans. The unpaired APTw CEST data at 2.0 μT using MTR asym showed a significant signal decrease at both C2 and C4 compared with baseline (P < .05), while the paired data showed a significant signal decrease only at C4 compared with baseline (P < .05). MTR asym at 0.9 μT did not show a statistically significant change between scans.

**Comparison of Signal Changes in pCR and Non-pCR**

Based on these results, Lor3.5 acquired using 0.9 μT and MTR asym acquired using 2.0 μT were selected for further comparisons between pCR and non-pCR groups for the 26 participants with pathology reports. The decrease in the group average Lor3.5 at 0.9 μT was significant at C2 compared with baseline for the pCR group (P = .03) (Fig 5A, Table E2 [supplement]). In contrast, the group average Lor3.5 change at 0.9 μT was not significant for the pCR group at baseline versus C4 and for the non-pCR group. The group average APTw CEST signal changes were not significant for either the pCR or non-pCR groups using the MTR asym with 2.0-μT saturation power (Fig 5C, Table E2 [supplement]). The APTw CEST signal changes on a per-participant basis at C2–baseline, C4–baseline, and C4–C2 for both Lor3.5 at 0.9 μT and MTR asym were compared with baseline for the pCR and non-pCR groups using an unpaired analysis (Fig 5B, Table E2 [supplement]).

**Figure 3**: The amplitude maps of the fitted Lorentzian line shapes at 3.5 ppm (Lor3.5) using (A, B) 2.0-μT saturation power and (C, D) 0.9-μT saturation power at baseline, after two cycles (C2), and after four cycles (C4) of the same participants as shown in Figure 2. The region of interest–averaged z-spectra (black circles, a plot of percent water signal against saturation frequency), the Lorentzian line shape fitting (red line), and the single Lorentzian line at 3.5 ppm (blue) of (A) and (C) are shown in (B) and (D), respectively. These results show that 0.9 μT should be used when evaluating Lor3.5. BL = baseline.
µT and MTR\text{asym} at 2.0 µT were compared between pCR and non-pCR groups (Fig 6). A decrease in the longest tumor diameter was observed for the pCR group at both C2 and C4 (Fig 6A, 6C). The decrease was significant at C4 compared with baseline. No significant decrease in the tumor diameter was found between time points in the non-pCR group. The longest tumor diameters were significantly different
between pCR and non-pCR groups at all time points. However, no significant differences were observed for the changes of the longest tumor diameters for either C2—baseline or C4—baseline between pCR and non-pCR groups (Fig 6B, 6C).

**Discussion**

In this study, we investigated APT\textsubscript{w} CEST MRI as an imaging marker for early treatment response in participants with TNBC receiving NAST by comparing two saturation power levels and two analysis methods. Our study demonstrated that the optimal CEST parameters depend on a proper pairing of the acquisition and analysis methods. For our study, the Lorentzian line shape fitting analysis method was better paired with acquisition at a low saturation power (0.9 \(\mu\)T), and the MTR\textsubscript{sym} analysis was better paired with acquisition at a high saturation power (2.0 \(\mu\)T). Using the appropriate combination of data acquisition and analysis methods, a significant APT\textsubscript{w} CEST signal decrease was observed in participants with TNBC after two cycles of NAST (\(P = .03\)).

The acquisition and analysis pairings for CEST MRI are intuitive. A high saturation power generates a higher contrast-to-noise ratio in the CEST MR images, favoring the asymmetry analysis, which is sensitive to noise in both the positive and negative parts per million range of the CEST spectrum (33). MTR\textsubscript{sym} is less favorable for analyzing the relatively noisy CEST spectra obtained from low saturation power. Conversely, the high saturation power broadens the CEST spectrum, which is then more difficult to quantify using Lorentzian line shape fitting analysis methods. For comparison, a low saturation power generates sharper CEST spectral features that can be evaluated with Lorentzian line shape analysis, but at the expense of lower contrast-to-noise ratio. Additionally, a lower saturation power can emphasize the magnetization transfer effect relative to the APT\textsubscript{w} CEST effect, which is mitigated by using Lorentzian line shape analysis that focuses on the APT\textsubscript{w} MRI signal at 3.5 ppm; this further emphasizes that Lorentzian line shape analysis is advantageous when low power saturation is applied. This finding is consistent with recommendations for the APT\textsubscript{w} MRI applied to brain imaging (13). Our finding contributes to clarifying the inconsistent results of previous APT\textsubscript{w} CEST MRI studies of breast cancer, which collectively have used all four combinations of MTR\textsubscript{sym} and Lorentzian line shape fitting methods to analyze CEST performed using low and high powers (18–25).

The signal decrease observed in participants when the appropriate combination of data acquisition and analysis methods was used can be attributed to a decrease in the concentration of mobile proteins and peptides in the tumor, which decreases the concentration of amide protons that can generate APT\textsubscript{w} CEST signal. Our results indicate that NAST decreases mobile protein concentration at an early stage of treatment in participants with either pCR or non-pCR, reflecting reduced protein expression as the TNBC is stressed by NAST. Importantly, this NAST-induced decrease in APT\textsubscript{w} CEST signal is not attributed to a change in tumor acidosis caused by treatment. In aggressive tumors, upregulated glycolysis is common, which causes the tumor to become acidic (34). The chemical exchange of an amide proton with water is base catalyzed; thus, CEST signal is low in acidic tumors, and CEST signal increases in response to treatments that reduce glycolytic metabolism. We did not observe an increase in APT\textsubscript{w} CEST in response to NAST, which indicates that the NAST-induced decrease in CEST is due to a decrease in mobile protein content, not to a decrease in glycolytic metabolism and tumor acidity. This result is similar to that of a previous study in which APT\textsubscript{w} CEST MRI was performed to evaluate the effects of breast cancer treatment, in which it also was concluded that the decrease in APT\textsubscript{w} CEST after treatment indicates a decrease in protein content rather than a decrease in acidosis (25).

A significant APT\textsubscript{w} CEST signal decrease was observed in pCR from baseline to C2 using 0.9-\(\mu\)T saturation power and Lorentzian line shape fitting. This is a major observation of our study, because the CEST signal change can be seen after only two cycles of NAST treatment, which can improve the evaluation of early response of TNBC to NAST. For comparison, no significant APT\textsubscript{w} CEST signal decrease was observed in the participants with non-pCR TNBC. However, no significant difference was observed between pCR and non-pCR groups in terms of CEST signal changes at any single time point using
either saturation power levels or analysis methods. This other major observation of our study shows that the change in CEST signal must be monitored during the course of NAST and cannot be evaluated at a single time point. In addition, these results demonstrate that APT$_w$ CEST MRI decreased in amplitude on a group average basis, which may not necessarily have value when assessing individual participants. Finally, the APT$_w$ CEST signals were not significantly different at C4 compared with baseline using all acquisition and analysis methods. We attribute this observation to the smaller tumor volumes at C4 in participants with either pCR or non-pCR, which may have reduced the precision of the ROI-averaged results from the pixel-by-pixel analyses if fewer imaging pixels are available for analysis. This result indicates that APT$_w$ CEST MRI may be limited for analyzing small tumors. This incentivizes the early assessment of NAST response in TNBC while the tumors remain relatively large. For comparison, the longest tumor diameter was significantly different between baseline and C4 for participants with pCR ($P < .001$), and it was significantly different for participants with pCR and non-pCR at C4 ($P < .01$), indicating that the longest tumor diameter is a better imaging feature than APT$_w$ CEST MRI at the C4 time point. These results are consistent with a previous APT$_w$ CEST MRI study of a murine model of glioblastoma (35).

There are limitations associated with this study. Although our CEST MR images showed no evidence of gross motion artifacts, presumably owing to the prone position of the participants, image registration could potentially improve the results, especially for participants with tumors located close to the chest wall that might be affected by breathing motion. Another limitation is that we acquired only single-section CEST scans. To acquire a CEST image set at the same location throughout the treatment, efforts were made to place the imaging sections for later time points as close as possible to the location used during the baseline scan. However, it was difficult to ensure that the imaging sections were at the same location, because the tumor often changed in volume during NAST. Therefore, a multisession or three-dimensional CEST imaging method that can image the entire tumor may potentially improve the results (36,37). Furthermore, multisession or three-dimensional CEST MRI could be used to investigate the spatial heterogeneity of APT$_w$ CEST MRI as an additional imaging marker of treatment response. A further limitation is that we tested only 0.9- and 2.0-μT saturation powers, based on a consensus recommendation to use 2.0-μT power for APT$_w$ CEST studies of brain cancer (13,38), as well as previous APT$_w$ CEST MRI studies of breast cancer that have used saturation powers in the range of 0.5 to 1.2 μT (18–25). Although future studies could test additional saturation powers, it is likely that such studies would reach the same conclusion drawn from this study that the acquisition and analysis methods should be carefully paired to provide optimal quantitative results from CEST MRI studies. We did not test the reproducibility of the APT$_w$ CEST MR scans owing to the workflow of our ongoing clinical trials; future studies might include reproducibility tests. However, other reports have shown good reproducibility of quantitative APT$_w$ CEST MRI applied to breast imaging studies (24,39). Finally, our study was performed at a single center. Our pilot study may spur additional studies at multiple centers, which may eventually address this limitation.

In summary, APT$_w$ CEST MRI acquisition and analysis methods should be appropriately combined to produce optimal results for response assessment. APT$_w$ CEST signal was shown to decrease in TNBC treated with NAST. The baseline–C2 change in this APT$_w$ CEST measurement may be a useful early response marker to help identify pCR among participants with TNBC following NAST. A similar decrease in APT$_w$ CEST was also observed for participants with non-pCR, however. Thus, distinguishing pCR from non-pCR status for an individual participant may be challenging.

Data sharing: Data generated or analyzed during the study are available from the corresponding author by request.

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