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***In vivo* ^1H MRS in the assessment of the therapeutic response of breast cancer patients**

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Abstract

MRI and *in vivo* MRS have rapidly evolved as sensitive tools for diagnosis and therapeutic monitoring in cancer research. *In vivo* MRS provides information on tumor metabolism, which is clinically valuable in the diagnosis and assessment of tumor response to therapy for the management of women with breast diseases. Several centers complement breast MRI studies with ^1H MRS to improve the specificity of diagnosis. Malignant breast tissues show elevated water-to-fat ratio and choline-containing compounds (total choline, tCho), and any effect of therapy on tissue viability or metabolism will be manifested as changes in these levels. Sequential ^1H MRS studies have shown significantly reduced tCho levels during the course of therapy in patients who were responders. However, there are challenges in using *in vivo* MRS because of the relatively low sensitivity in detecting the tCho resonance with decreased lesion size or significant reduction in the tumor volume during therapy. MRS is also technically challenging because of the low signal-to-noise ratio and heterogeneous distribution of fat and glandular tissues in the breast. MRS is best utilized for the diagnosis of focal masses, most commonly seen in patients with ductal-type neoplasms; however, it has limitations in detecting nonfocal masses, such as the linear pattern of tumors seen in invasive lobular carcinoma. Further work is required to assess the clinical utility of quantitative MRS, with the goal of automation, which will reduce the subjectivity currently inherent in both qualitative and semi-quantitative MRS.

Keywords

MRS; breast cancer; therapeutic response; total choline (tCho); water-to-fat (W-F) ratio; signal-to-noise ratio (SNR); quantification; tumor volume

INTRODUCTION

Breast cancer is the most common malignancy among women and is a significant cause of mortality and morbidity (1,2). Early diagnosis of malignancy and early treatment are

essential to improve the quality of life of breast cancer patients and their survival. In this regard, radiologic techniques play a crucial role in the screening, diagnosis, image-guided biopsy and assessment of the therapeutic response of patients in a clinical setting. Typically, neoadjuvant chemotherapy (NACT) is the standard treatment option for patients with advanced-stage disease or locally advanced breast cancer (LABC), which is followed by surgery and post-operative therapies (3,4). NACT offers several advantages for patients with LABC, such as a reduction in tumor size, option of breast conservation surgery and inhibition of distant metastases (3,5–7). Several clinical trials have shown that the degree of response of the primary tumor to pre-operative chemotherapy correlates with patient survival (8–10). However, NACT has drawbacks because of the toxicity of drugs and the variation in response of individual patients. It has been reported that the overall response rates to chemotherapy vary from 69% to 100% in various clinical trials, with complete pathologic response rates of only 10–31% (6,7). It is therefore essential to identify nonresponders so that they may be offered alternative forms of treatment. Thus, the early and accurate assessment of treatment response is an essential part of breast cancer patient management.

The most common method for assessing a patient's treatment response to therapy in the clinical setting is the physical examination of the palpable change in the tumor size. Mammography and sonography are frequently used for the evaluation of breast malignancy; however, they are not accurate in differentiating between chemotherapy-induced fibrosis and residual tumor (3,11–13). Breast MRI is useful for clinical staging and also for the measurement of residual disease by determining the size of the tumor both prior to and after therapy (14–16). Several studies have reported that dynamic contrast-enhanced MRI is more effective than mammography, sonography or clinical examination for the estimation of residual disease following chemotherapy (17–20). However, it has limitations, as anti-angiogenic treatment may lead to decreased contrast uptake and thereby reduce the signal enhancement. Residual disease may also be missed in diffuse tumors because of partial volume averaging. Rieber *et al.* (21) showed that MRI often tends to underestimate the amount of tumor, relative to histology, especially in tumors that respond well to chemotherapy. The role of MRI in the assessment of the breast cancer response to NACT has recently been reviewed by Hylton (15). MRI studies on the assessment of residual disease following chemotherapy reported both under- and overestimation of residual disease compared with pathologic assessment (15).

The imaging methods described above are based on the measurement of changes in tumor size for the assessment of the tumor response; however, these are evident only at later stages of therapy. For example, it has been reported that changes in lesion size on dynamic contrast-enhanced MRI may not be detected until after two cycles of chemotherapy (21). Thus, if an early surrogate response indicator can be established to predict the final treatment outcome, it would benefit patients. In this direction, techniques that measure the biochemical and functional activity of the tumor may show promise in the early prediction of response with higher accuracy by detecting the effects of therapy on tumor biology rather than on structural changes. In this context, the utility of diffusion-weighted imaging (DWI) and *in vivo* MRS has been explored, in particular with regard to the evaluation of the early

response of breast cancer to therapy. Recently, the apparent diffusion coefficient (ADC) of tumors measured using DWI has been shown to predict the early response relative to anatomical parameters (22,23).

In the last decade, considerable interest has been shown in the use of ^1H MRS methods for the differential diagnosis of benign and malignant breast lesions and for monitoring the therapeutic response of breast tumors (24,25). Tissues are not only rich in water and fat, but also in many biochemicals (metabolites) that are detected by MRS. The MR signal intensity, intensity ratios and concentrations of metabolites determined by MRS are valuable for the differentiation of malignant, benign and normal tissues. Furthermore, the monitoring of changes in these parameters during therapy provides valuable information on the tumor response to different therapeutic regimens in a noninvasive manner. The assumption is that malignant breast tissues are characterized by a high water content and elevated levels of choline-containing compounds (total choline, tCho), and any effect of therapy on tissue viability or metabolism would be manifested as changes in the water-to-fat (W-F) ratio or tCho levels (24–51). Several centers complement breast MRI studies with MRS to improve the specificity, which has been reported to be around 88% from various studies (27). In this article, we review the potential of *in vivo* ^1H MRS in the clinical setting for the monitoring of the therapeutic response of breast cancer patients.

BRIEF EXPERIMENTAL DETAILS OF BREAST CANCER IN VIVO MRS

Most *in vivo* breast ^1H MRS studies reported in the literature to date were carried out at 1.5 T; however, recently, several investigations at high fields have also been reported (25,45). Generally, the patient is positioned prone with the breasts fitting into a double breast volume surface coil for maximum signal reception. The body coil is used as the transmitter. Following localizer ^1H MRI, T_1 -weighted, T_2 -weighted and fat-saturated high-resolution images are acquired in different planes. Dynamic contrast-enhanced MRI is preferred for appropriate positioning of a voxel of an appropriate size that usually depends on the tumor size. As breast lesions are heterogeneous, the detection and level of tCho are sensitive to the size and placement of the voxel (24,25,30). *In vivo* ^1H MR spectra are acquired either from a single voxel (SV) or from multiple small voxels using chemical shift imaging or multi-voxel MRSI. Both global and voxel-level shimming are essential to optimize the magnetic field homogeneity, which is important for good water and/or water + fat suppression. In breast ^1H MRS studies, the typical linewidth achieved for water resonance is around 5–15 Hz, whereas, by shimming on the fat peak, a linewidth of 10–25 Hz is achieved (as in the case of normal breast tissue). Both unsuppressed and water + fat-suppressed spectra are acquired using different TEs (30, 135, 270 and 350 ms). Spectra are referenced with respect to water as the internal standard at 4.7 ppm. The age and menopausal status of the patient should be taken into account during the interpretation of the data, as post-menopausal women have more adipose tissue, relative to glandular tissue in pre-menopausal women, especially when the water-to-fat ratio (W-F ratio) is used for response assessment.

W-F RATIO

A typical *in vivo* ^1H MR spectrum (without water and fat suppression) obtained from the normal breast tissue of a volunteer is shown in Fig. 1B (the voxel position from which the spectrum was obtained is shown in Fig. 1A), and is dominated by fat (lipid) at 1.33 ppm (methylene $[-(\text{CH}_2)_n]$ protons) with a water peak at 4.7 ppm. From the respective peak areas in the unsuppressed spectrum, the W-F ratio can be calculated. Figure 2 shows the MR image and ^1H MR spectrum obtained with and without water + fat suppression from a patient suffering from LABC (infiltrating ductal carcinoma). Two important parameters are measured from the *in vivo* spectrum of malignant breast tissue: (i) W-F ratio from the unsuppressed spectrum (Fig. 2B); and (ii) tCho concentration from the suppressed spectrum (Fig. 2C). In malignant breast tissue, the water resonance dominates with high W-F ratio compared with normal breast tissue (26,32–34).

Table 1 summarizes the various ^1H MRS studies reported in the literature to monitor the therapeutic response of breast cancer patients. Jagannathan *et al.* (32) evaluated the use of the W-F ratio as a response indicator in 44 patients with LABC; 17 of the 44 patients were evaluated prior to therapy and their W-F ratio was 6.0 ± 6.9 . In 11 patients who were monitored after one to two cycles of NACT, the W-F ratio was 4.5 ± 4.4 , whereas it was 1.4 ± 1.1 in 13 patients who were monitored after three to four cycles of therapy. This value reduced further to 1.3 ± 1.5 in 19 patients monitored after five to six cycles of NACT. Later, Kumar *et al.* (34) conducted a controlled serial *in vivo* SV ^1H MRS study and evaluated the utility of the W-F ratio and tumor volume as response indicators in patients with LABC. Sequential ^1H spectra both prior to and after the completion of NACT were acquired in 28 patients at 1.5 T. Prior to therapy, a statistically significantly higher W-F ratio ($p < 0.01$) was observed in malignant breast tissues compared with controls. In patients receiving NACT, a significant decrease ($p < 0.01$) in the W-F ratio (see Fig. 3), together with a reduction in the primary tumor size, was observed. In addition, the pre-therapy W-F ratio showed a fair correlation with the pre-therapy tumor volume ($p = 0.05$), but the post-therapy W-F ratio showed no such correlation with the post-therapy tumor volume. The observed trend in the reduction of the W-F ratio provided a noninvasive response indicator to monitor the clinical outcome of breast cancer patients given NACT (34).

Manton *et al.* (36) also reported the W-F ratio in women with LABC undergoing NACT, and the experiments were carried out before treatment and after the second cycle of chemotherapy. They reported that early changes in the W-F ratio and water T_2 (after the second cycle of therapy) have substantial prognostic efficacy, whereas the pharmacokinetic parameters and ADC did not detect the early response. In 50% of cases (3/6), the W-F ratio accurately predicted nonresponse with 100% sensitivity and a negative predictive value. The authors concluded that this level of accuracy might permit clinical application, where an early and accurate prediction of nonresponse would permit an early change to second-line treatment, thus sparing patients the unnecessary toxicity, psychologic morbidity and delay of initiation of effective treatment.

The studies cited above demonstrate that simple W-F ratio determination from conventional *in vivo* SV ^1H MRS is useful for monitoring the therapeutic response of breast cancer

patients. However, the W–F ratio has certain limitations as it is not useful for the characterization of lobular carcinoma and other types of breast tumor. Furthermore, one of the difficulties in calculating the W–F ratio arises because of a dynamic range problem in detecting these resonances when there is excessive water and a significant reduction in fat, which occurs in the case of malignant tumors. Thus, it is important to explore other biochemical markers that would serve as better predictors of response assessment.

TOTAL CHOLINE (tCho)

Qualitative assessment

Many research groups have reported a unique peak at 3.2 ppm corresponding to free choline, phosphocholine and glycerophosphocholine. This peak is designated as tCho and has been established as a reliable biochemical marker of breast malignancy, and also as a marker of response assessment of breast cancer patients (24,25,27–32,35,37–51). Kvistad *et al.* (28) reported the first observation of changes in the tCho signal in response to therapy in one subject undergoing NACT. In their study, the tCho peak that was observed prior to therapy was absent after treatment. Later, Jagannathan *et al.* (35) reported a more systematic study using the tCho resonance to assess the therapeutic response to chemotherapy in 67 patients with LABC using SV ¹H MRS. Thirty-two patients (Group I) were investigated only prior to therapy (1 week before NACT) and the tCho resonance was observed in 81%. Group II consisted of 21 patients who were investigated 1 week after the completion of the third or sixth cycle of NACT, and tCho was absent in 86% (18/21). Sequential monitoring was carried out on 14 patients (Group III) both prior to therapy and 1 week after the third or sixth cycle of NACT. In this group, tCho was observed in 10 of the 14 cases before treatment. Of these 10 patients, seven showed no or significantly reduced tCho, indicating a response to NACT, as evidenced by clinical and histopathologic evaluation. Histopathologic evaluation showed three patients to be nonresponders, whereas, in MRS, the tCho signal was absent in two patients and, in one patient, a reduction in tCho was observed. Thus, post-therapy histopathologic investigation of Group III patients correlated well with the presence or absence of the tCho peak. This study showed that the observation of tCho before treatment and its disappearance (or reduction) after treatment may be a useful indicator of the response of patients with LABC to NACT.

The above studies reported the qualitative assessment of the tCho resonance by observing its presence or absence (including reduction). However, the quantitative estimate of the absolute concentration of tCho provides a more objective assessment of the variation of tCho levels as a result of therapy. Furthermore, a quantitative estimate of tCho is necessary to compare measurements with potentially different sensitivities, especially in serial studies in which tumor regression occurs as a result of therapy. There are two commonly used approaches: (i) semi-quantitative estimation of tCho by calculating the tCho integral and the signal-to-noise ratio (SNR) of the choline signal; and (ii) determination of the absolute concentration of tCho using the tissue water resonance as an internal or external reference.

Choline signal-to-noise ratio (ChoSNR)

There are few studies that have reported the use of the semi-quantitative assessment of ChoSNR for the differentiation of malignant and benign lesions, as well as for monitoring the therapeutic response (37–42). ChoSNR is measured using the peak height (intensity) of the tCho signal and the intensity of noise from the off-resonance region of the ^1H MR spectrum with the formula: $\text{ChoSNR} = \text{amplitude of tCho resonance} / \text{root-mean-square amplitude of noise}$. Tozaki *et al.* (40) reported the assessment of the efficacy of the tCho integral in the evaluation of the early response to NACT in seven breast cancer patients, and compared it with the standardized uptake value of ^{18}F -fluorodeoxyglucose using positron emission tomography. The mean integral of tCho was 2.5 (range, 1.2–5.3) before chemotherapy. Three of seven patients did not show tCho resonance and were also negative for the standardized uptake value after two cycles of chemotherapy, and one patient became negative before surgery. In the remaining three patients, the curves of both parameters (tCho and the standardized uptake value) paralleled the time course of chemotherapy treatment, and the reduction rate of both the tCho integral and the standardized uptake value after chemotherapy showed a correlation ($r = 0.84$, $p = 0.02$).

Recently, the same group (41) reported the use of the tCho integral and ADC derived from DWI in the evaluation of the early response to NACT in nine patients. MR studies were carried out both prior to therapy and after the first cycle of NACT. Pre-therapy MRS demonstrated the presence of tCho in six cases. DWI before therapy demonstrated a localized high-signal lesion in all cases. The change in the integral value of the tCho resonance after the first cycle of NACT showed a positive correlation with the change in lesion size, whereas no such correlation was observed between ADC and the lesion size after the first cycle of NACT. The authors concluded that MRS after the first cycle of therapy may be more sensitive than DWI for the prediction of the pathologic response.

Recently, Danishad *et al.* (42) reported systematic changes in ChoSNR using MRSI and the anatomical parameter (tumor volume) in 30 patients with LABC, and evaluated the potential clinical utility of these parameters in the assessment of tumor response during the various stages of NACT. They carried out sequential monitoring at four time points, namely prior to therapy and after the first, second and third cycles of NACT, and ChoSNR and the tumor volume were measured. The MR response was compared with the clinical response. Typical ^1H MRSI spectra acquired prior to and after therapy from a responder and a nonresponder are shown in Figs. 4 and 5 (42).

ChoSNR showed a significant reduction in responders following NACT compared with the pre-therapy value. The percentage reductions in ChoSNR were $27.8 \pm 7.8\%$ after the first, $48.8 \pm 17\%$ after the second and $93.5 \pm 9.4\%$ after the third cycles of NACT. Of the 14 responders, 10 did not show tCho resonance after the third cycle of NACT, whereas, in the remaining four patients, the mean ChoSNR was reduced to a value of $3.6 \pm 1.1\%$ ($n = 4$). In nonresponders, the mean percentage changes in pre-therapy ChoSNR were $8.6 \pm 3.9\%$ after the first, $16 \pm 11\%$ after the second and $38.5 \pm 19\%$ after the third cycles of NACT.

Further, Danishad *et al.* (42) reported cut-off values for the percentage change in ChoSNR and volume after the third cycle of NACT to predict the tumor response. Decreases of 53%

in ChoSNR and 47.5% in volume were reported as the cut-off percentages to differentiate responders from nonresponders. The sensitivity of ChoSNR for the detection of responders was 85.7%, with 91% specificity to differentiate nonresponders. The volume showed 100% sensitivity with 73% specificity. ChoSNR and volume showed a correlation of $r = 0.7$ after the third cycle of NACT. These results demonstrate that ChoSNR may serve as a useful parameter to differentiate responders from nonresponders with higher specificity relative to volume, suggesting its potential in the effective treatment management of breast cancer patients. The advantage of the multi-voxel technique is that it provides a wide coverage of the organ of interest and allows the monitoring of the changes in ChoSNR simultaneously from the tumor area as well as from the normal portion of the breast. However, the SNR method has some limitations in that noise is affected by factors related to the instrument, patient movement, etc. These can be partially eliminated by keeping the voxel size constant during the study, which is the major source of variation. In addition, there are other limitations in performing breast chemical shift imaging that arise from the heterogeneity of breast tissue, relative to brain tissue which is more homogeneous.

Quantitative assessment of tCho concentration

Although semi-quantitative estimates of tCho using ChoSNR have been found to be useful in evaluating the therapeutic response of patients with breast cancer, quantitative estimates provide an objective assessment of the variation in the tCho signal as a result of therapy. However, because of the heterogeneous distribution of glandular and adipose tissues in breast, the quantification of metabolite levels is more difficult than that in the brain. Generally, the absolute quantification of metabolites is carried out using a reference signal. Two referencing methods have been developed for the conversion of the measured tCho signal amplitude into concentration: (i) internal referencing, in which tissue water is used as a standard; and (ii) external referencing, in which an *in vitro* substance phantom (e.g. phosphocholine chloride solution) is used as a standard (25,43,45). Methods using water as external or internal reference have also been developed (25,43,45). The internal reference method using the water resonance overcomes some of the limitations of the external reference method, such as the need for separate calibration experiments and correction for partial volume effects. MR studies carried out at 4.0 T reported tCho concentrations in the range 0.0–10.5 mmol/kg (25,45), whereas, at 1.5 T, the concentrations were reported to be in the range 0.21–0.76 mmol/kg (43,44,46–52). A wide range of tCho concentrations for malignant lesions has been reported by various researchers (25,43–52). The large range of tCho concentrations observed in these studies may reflect the heterogeneous nature of breast lesions. For example, tCho detection may be difficult in diffuse enhancement-type cancers because of the intermingling of tumor cells with adipose tissues. Diffuse enhancement-type cancer shows a much lower overall tCho level than mass-type cancer ($p = 0.035$) (52). In addition to the intrinsic heterogeneous nature of breast tumors, the limitations of *in vivo* ^1H MRS detection may also contribute to the large variation in the tCho distribution.

A pilot study using quantitative ^1H MRS in 13 patients with LABC treated with doxorubicin-based NACT was reported by Meisamy *et al.* (45) at 4 T. MRS was carried out prior to treatment and 1 day after the first cycle of NACT, and again at the end of the chemotherapy regimen. A significant change in tCho concentration at day 1 was reported by

the authors, which was different between responders and nonresponders ($p = 0.007$). Furthermore, a strong positive correlation was reported between the change in tCho concentration (from baseline to 1 day after the first dose of chemotherapy) and the change in lesion size. The change in lesion size was measured at the end of the fourth cycle of chemotherapy. This study demonstrated that the decrease in tCho concentration measured on day 1 can serve as an early indicator of clinical response. In a recent study, Haddadin *et al.* (25) reported the data of 28 patients obtained at 4 T, and found that 75% of the responders showed a decrease in tCho at day 1 post-therapy, whereas 92% of nonresponders showed no change or an increase in tCho concentration at day 1 after therapy.

Tan *et al.* (46) investigated 18 patients with invasive ductal carcinoma at 1.5 T during the course of NACT. Patients were scanned before treatment, 1–8 days after the first cycle of NACT and, thereafter, 2–3 weeks after the second and last NACT using an SV ^1H MR TE-averaging method. The concentration of tCho was determined using LCmodel corrected for relaxation *in vivo*. The authors observed no statistically significant differences between responders and nonresponders in either the baseline tCho levels or changes in tCho 1–8 days after the first cycle of therapy. Sah *et al.* (47) reported the clinical potential of *in vivo* SV ^1H MRS by calculating the absolute concentration of tCho both prior to and during various stages of NACT in 17 patients with LABC. They observed a significant reduction in the tCho concentration as early as after the first cycle of NACT in responders compared with nonresponders. Further reduction was observed after the second and third cycles of NACT (47). The changes in tCho concentration and tumor size during NACT between patients who showed pathologic complete response (pCR) and those who did not (non-pCR) was reported by Baek *et al.* (48). tCho before treatment was seen in 14 of 16 patients who were responders. At baseline, the tCho concentration was similar in responders (2.30 ± 2.24 mmol/kg) and nonresponders (2.51 ± 2.27 mmol/kg). In responders, an average reduction of 50% was observed in tCho level after one or two cycles of therapy and the mean percentage change in lesion size was – 18%. In nonresponders, the mean percentage change in tCho was – 14% and the mean change in lesion size was – 15% after one or two cycles of chemotherapy. This study showed that early metabolic changes were greater than the changes in tumor size. The early reduction in tCho was interpreted as reflecting the inhibition of cellular proliferation and the cytotoxic effect of chemotherapy (48).

In another study of 35 breast cancer patients, Baek *et al.* (49) compared changes in the tCho concentration and tumor size at follow-up after NACT between patients who showed pCR and non-pCR. The treatment included doxorubicin and cyclophosphamide, followed by a taxane-based regimen. They reported that the changes in tCho level were greater than the changes in tumor size in the pCR group in both the first follow-up (after the first or second cycle) and second follow-up (after the third or fourth cycle) studies relative to the non-pCR group. The authors concluded that patients who showed a greater reduction in tCho concentration compared with the change in tumor size were more likely to achieve pCR, and the change in tumor size halfway through therapy was the most accurate predictor of pCR.

Tozaki *et al.* (50) reported the efficacy of quantitative ^1H MRS using the external standard method to predict the pathologic response in 16 breast cancer patients subjected to NACT with an anthracycline-based regimen. MRI and MRS were performed before therapy and

after the second cycle of NACT, and the tumor response was evaluated. The final histopathologic findings following surgery after the fourth cycle of NACT were used as reference. There were no significant differences in the baseline tumor size and normalized tCho signals between pCR ($n = 8$) and non-pCR ($n = 8$). The reduction rates of the normalized tCho signal were statistically significantly different between the pathologic responders and nonresponders, whereas the reduction rates of the lesion size were not significantly different between the two groups. When a reduction rate of 40–50% of the normalized tCho value was chosen as the cut-off, the positive and negative predictive values of MRS were 89% (8/9) and 100% (7/7), respectively. It was concluded that the change in tCho after the second cycle of NACT may be more sensitive than the change in tumor size for the prediction of the pathologic response.

Recently, Sah *et al.* (51) have reported that the tCho concentration is a better predictor than the tumor volume of the early response of breast cancer. Thirty patients with LABC were evaluated by sequential MRI and *in vivo* SV ^1H MRS prior to and during various stages of NACT. The pre-therapy concentration of tCho showed a significant reduction as early as after the first cycle of NACT in responders relative to nonresponders. Further reduction was observed after the second and third cycles of NACT in responders. The tumor volume showed a significant decrease only after the second cycle of NACT in clinical responders. Typical ^1H MR spectra acquired prior to and after therapy from a responder are shown in Fig. 6.

SUMMARY AND FUTURE DIRECTIONS

The various studies reported to date in the literature indicate that *in vivo* ^1H MRS is a promising tool for the characterization of the response of breast tumors to therapy in the management of women with breast disease by facilitating the assessment of treatment efficacy. Combined with water and fat suppression, it is possible to obtain ‘noninvasive biopsy’ biochemical information from the measurement of endogenous metabolites, such as tCho, which reflect the changes in tissue metabolism; this is useful not only for diagnosis, but also for monitoring the tumor response to therapy by repeated evaluation of patients during treatment.

However, the currently available data show variable findings with regard to the efficacy of breast *in vivo* ^1H MRS for the monitoring of the tumor response to therapy. These may arise from methodologic differences across studies, such as the field strength, coil, sequences and variation in the protocol used. As different therapeutic drug regimens were used in these studies, it is probable that the response and the induced changes in tCho may be intrinsically different. Moreover, there was no consistency in the time points at which the post-treatment MRS examinations were carried out in these studies (25,32–36,42,44–49,51), which might also have led to variable findings. The actual timing at which MRS can be carried out during therapy may also play a crucial role. For example, monitoring by MRS early during therapy may not affect the sensitivity of MRS, as anatomical changes are expected to be small, as are the physiologic changes that can confound tCho measurements. Well-controlled large-scale studies are therefore required to determine the actual time point to carry out post-therapy MRS after the initiation of therapy. Another challenge is the low sensitivity of MRS

in detecting the tCho resonance during therapy because of various factors, such as the decreased lesion size or total disappearance of the tumor.

Previously, NACT was used mainly to decrease the size of inoperable cancers and render them operable. However, as patients with operable cancer receiving NACT followed by surgery showed an equivalent prognosis to patients receiving surgery followed by adjuvant chemotherapy, NACT is now being widely used for patients with operable breast cancer (53). Not only does NACT facilitate breast conservation surgery, it has also been shown that patients who achieve pCR have a favorable prognosis (54). It has also been shown that, if patients do not achieve pCR, but have only a minimal residual tumor burden remaining in the breast, they also have the same prognosis as pCR patients (55).

In this review, we have presented a summary of the ^1H MRS studies that have been reported in the literature on the assessment of tumor response to therapy in breast cancer patients. It should be noted that many studies were designed to differentiate between clinical responders and nonresponders. As more chemotherapeutic agents and targeted agents (e.g. trastuzumab for targeting HER-2-positive cancer and bevacizumab for targeting angiogenesis) have become available for the treatment of breast cancer, the response rate has been steadily improving. Today, most patients will respond to the current therapy to some extent, so that rarely will a patient be classified as a nonresponder. For future studies, investigators should be encouraged to predict the prognosis of patients based on their pathologic responses rather than their clinical responses. Furthermore, as the oncology field has not reached a consensus about how to categorize patients who have minimal residual disease, they should be separated from patients who have residual bulk disease and patients who have achieved pCR in the initial analysis (56–58). Later, the groups may be combined to evaluate the predictive value of MRS for pathologic response after NACT.

The future of *in vivo* breast MRS rests on improved sensitivity with increased SNR using specialized coils and high fields (these will provide high-quality spectra because high field strengths yield better spectral resolution). With the wide availability of 3 T MR scanners, the detection of tCho should improve. Further improvements should be focused on new and improved pulse sequences for faster data acquisition, robust quantitative methods for the determination of the absolute concentrations of metabolites from both SV and MRSI methods, and the use of two-dimensional spectroscopy in clinical settings for improved spectral assignment of metabolites and fat (59). Furthermore, MRI also offers the capability to evaluate the cellular changes using DWI and vascular changes using dynamic contrast-enhanced MRI in the same imaging session as MRS. Together with the metabolic changes evaluated by MRS, they may allow the development of a multi-parametric imaging classifier to predict the pathologic therapeutic response, which should achieve a higher accuracy compared with the use of either set of imaging parameters alone.

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Abbreviations used

ADC	apparent diffusion coefficient
ChoSNR	choline signal-to-noise ratio
DWI	diffusion-weighted imaging
LABC	locally advanced breast cancer
NACT	neoadjuvant chemotherapy
non-pCR	no pathologic complete response
pCR	pathologic complete response
SNR	signal-to-noise ratio
SV	single voxel
tCho	total choline
W-F ratio	water-to-fat ratio

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Figure 1.

(A) MRI showing the voxel position from which the single-voxel (SV) ^1H MR *in vivo* spectrum (B) was obtained from the normal breast tissue of a volunteer [without water and fat (lipid) suppression].

Figure 2.

(A) MRI of a patient with locally advanced breast cancer showing the voxel position from which the single-voxel (SV) ^1H MR *in vivo* spectrum was obtained without water and fat (lipid) suppression (B) and with the suppression of water + fat (lipid) resonances (C).

Figure 3.

Graph showing an individual patient's pre- and post-therapy water-to-fat (W-F) ratios.
 [Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.,
 from ref. (34).]

Figure 4.

(A) Pre-therapy T_2 -weighted sagittal fat-suppressed image of a patient with locally advanced breast cancer who was a responder with the MRSI grid. (B) Spectrum obtained from a voxel highlighted in (A) showing the total choline (tCho) signal. (C) Post-therapy MRI of the same patient after the third cycle of neoadjuvant chemotherapy (NACT). (D) Spectrum obtained from a voxel highlighted in (C) that showed no tCho. [Reproduced with permission from John Wiley & Sons from ref. (42).]

Non-Responder

Pre-therapy

Post-therapy

Figure 5.

(A) Pre-therapy T_2 -weighted sagittal fat-suppressed image of a patient with locally advanced breast cancer who was a nonresponder with the MRSI grid. (B) Spectrum obtained from a voxel highlighted in (A) showing the total choline (tCho) signal. (C) Post-therapy T_2 -weighted sagittal fat-suppressed image of the same patient after the third cycle of neoadjuvant chemotherapy (NACT). (D) Spectrum obtained from a voxel highlighted in (C) showing the tCho signal. [Reproduced with permission from John Wiley & Sons from ref. (42).]

Figure 6.

(A) Pre-therapy T_2 -weighted sagittal fat-suppressed image of a patient with locally advanced breast cancer who was a responder, showing the voxel from which the single-voxel (SV) ^1H MR spectrum (B) was obtained. (C) Post-therapy T_2 -weighted sagittal fat-suppressed image of the same patient, showing the voxel from which the ^1H MR spectrum (D) was obtained with reduced total choline (tCho) concentration.

Table 1

Summary of ¹H MRS studies reported in the literature on breast cancer patients monitored during therapy

Reference	Field (T)	Method and sequence; TR/TE	Voxel size (cm ³)	Biomarker used	Number of patients (n) studied and time point of MRS	Findings
Jagannathan <i>et al.</i> (32)	1.5	SV, STEAM; TR/TE = 3000/135 ms	1–8	W–F ratio	n = 44; split into 4 groups Group 1 (n = 17); MRS at Tp0 Group 2 (n = 11); MRS at Tp1 and Tp2 Group 3 (n = 10); MRS at Tp3 and Tp4 Group 4 (n = 13); MRS at Tp6	Elevated W–F ratio in malignant tissues; no significant change in W–F ratio after Tp1 and Tp2; significant reduction after Tp3 onwards
Kvistad <i>et al.</i> (28)	1.5	SV; PRESS; TR/TE = 2000/135, 350, 450 ms	1–25.2	tCho peak (presence or absence of the peak)	n = 1; sequential study with MRS at Tp0 and after completion of NACT	No tCho peak seen at the end of chemotherapy
Jagannathan <i>et al.</i> (35)	1.5	SV; STEAM; TR/TE = 3000/135 ms	1–8	tCho peak (presence or absence of the peak)	n = 67 (in 3 groups) Group 1 (n = 32); MRS at Tp0 only Group 2 (n = 21); MRS at Tp3 or Tp6 only Group 3 (n = 14); sequential study with MRS at Tp0 and Tp3 or Tp6	tCho seen in 3/21 in Group 1. In Group 3, 10/14 showed tCho at Tp0. After NACT, tCho peak reduced or absent in 7/10 cases
Meisamy <i>et al.</i> (45)	4.0	SV; LASER; TR/TE = 3000/45–196 ms	Depending on the tumor size	tCho concentration	n = 16; sequential study with MRS at Tp0 and after 24 h of Tp1 and at Tp4	Data analyzed for n = 13; 8 of 13 objective responders showed change in tCho concentration after 24 h compared with baseline (Tp0)
Manton <i>et al.</i> (36)	1.5	MRSI; STEAM multivoxel; TR/TE = 3000/135 ms		W–F ratio	n = 22; sequential study with MRS at Tp0 and Tp2	W–F ratio predicted nonresponse in 50%, and sensitivity was 100%
Kumar <i>et al.</i> (34)	1.5	SV; STEAM; TR/TE = 3000/135 ms	1–8	W–F ratio	n = 33; sequential study with MRS at Tp0 and Tp3 or Tp6	W–F ratio showed significant decrease after therapy. In responders, the sensitivity was 87% with 79% accuracy. In nonresponders, the sensitivity was 70% with 71% accuracy
Tan <i>et al.</i> (46)	1.5	TE averaging SV; sequence not mentioned; TR/TE = 1500/35 and 2000/145 ms	0.56–11.6	tCho concentration	n = 18; sequential study with MRS at Tp0 and Tp2 and Tp4 or Tp6	The conclusion of the study was that monitoring of tCho does not appear to be a robust method of predicting response to therapy
Tozaki <i>et al.</i> (40)	1.5	SV; PRESS; TR/TE = 1620/270 ms	3.4	tCho integral	n = 7; sequential study with MRS at Tp0 and Tp2	tCho integral reduced in responders
Baek <i>et al.</i> (48)	1.5	SV; PRESS; TR/TE not mentioned	2.4–8.0	tCho concentration	n = 20; sequential study with the first follow-up MRS at Tp0 and after Tp1 and Tp2; second follow-up MRS at Tp0 and Tp4	Percentage change in tCho was 50% after first follow-up and the change in lesion size was 18%
Sah <i>et al.</i> (47)	1.5	SV; PRESS; TR/TE = 1500/100 ms	Depending on the tumor size	tCho concentration	n = 15; sequential study with MRS at Tp0, Tp1, Tp2 and Tp3	Changes in tCho concentration after Tp1 compared with Tp0
Baek <i>et al.</i> (49)	1.5	SV; PRESS; TR/TE = 2000/270 ms	2.4–8.0	tCho concentration	n = 35; sequential study with the first follow-up MRS at Tp0 and after Tp1 and	Percentage change in tCho was greater than the change in tumor size. At first follow-up

Reference	Field (T)	Method and sequence; TR/TE	Voxel size (cm ³)	Biomarker used	Number of patients (n) studied and time point of MRS	Findings
Haddadin <i>et al.</i> (25)	4.0	SV; LASER; TR/TE and other parameters used as in ref. (45)	Depending on the tumor size	tCho concentration	Tp2; second follow-up MRS at Tp0 and Tp4 no significant difference between pCR and Tp2; second follow-up MRS at Tp0 and Tp4 non-pCR groups <i>n</i> = 28; sequential study with MRS at Tp0, after 24 h of Tp1 and at the end of therapy	75% of objective responders showed decreased tCho after 1 day of treatment, and 92% of nonresponders showed no change or an increase in tCho
Danishad <i>et al.</i> (42)	1.5	PRESS MRSI (2D); multivoxel; TR/TE = 2000/135 ms		tCho SNR	<i>n</i> = 30; sequential study with MRSI at Tp0, Tp1, Tp2 and Tp3	In responders, a significant reduction in ChoSNR was seen at Tp1 and thereafter. Volume showed significant reduction only at Tp2
Tozaki <i>et al.</i> (41)	1.5 T	SV; PRESS; TR/TE = 1620/270 ms	3.4	tCho integral	<i>n</i> = 9; sequential study on 7 patients only; MRS at Tp0 and Tp1	Changes in tCho integral showed positive correlation with lesion size. MRS after Tp1 may be more sensitive than DWI to predict response
Tozaki <i>et al.</i> (50)	1.5	SV; PRESS; TR/TE = 1620/270 ms	3.4	tCho concentration	<i>n</i> = 34; sequential study on 16 patients only; MRS at Tp0 and Tp2	Change in tCho concentration after Tp2 more sensitive than the tumor size for monitoring tumor response
Sah <i>et al.</i> (51)	1.5	SV; PRESS; TR/TE = 1500/100 ms	Voxel size used depending on the tumor size	tCho concentration	<i>n</i> = 30; sequential study with MRS at Tp0, Tp1, Tp2 and Tp3	In clinical responders, changes in tCho concentration were observed after Tp1, whereas tumor volume showed changes only after Tp2

DWI, diffusion-weighted imaging; LASER, localization by adiabatic selective refocusing; non-pCR, nonpathologic complete response; pCR, pathologic complete response; PRESS, point-resolved spectroscopy; SNR, signal-to-noise ratio; STEAM, stimulated echo mode; SV, single voxel; tCho, total choline; Tp0, pre-therapy; Tp1, after first cycle of therapy; Tp2, after second cycle of therapy; Tp3, after third cycle of therapy; Tp4, after fourth cycle of therapy; Tp6, after sixth cycle of therapy; W-F ratio, water-to-fat ratio.