

# Breast tissue characterization by *in vivo* Magnetic Resonance Spectroscopy (MRS)

N.R. Jagannathan \*

*Department of NMR and MRI Facility, All India Institute of Medical Sciences, New Delhi, India*

**Abstract.** *In vivo* magnetic resonance spectroscopy (MRS) is a sensitive technique for metabolic evaluation of normal, benign and malignant tissues and is rapidly evolving as a clinical tool for diagnosis, characterization and monitoring the therapeutic response of tumor. The proton ( $^1\text{H}$ ) MR spectra of normal breast tissues are characterized by high fat content while tumor tissues have higher water content. From the respective peak areas, the water–fat (W–F) ratio can be calculated. The *in vivo*  $^1\text{H}$  MR spectrum obtained with water and fat suppression from malignant breast tissues are characterized by a peak at 3.2 ppm corresponding to choline-containing metabolites (tCho) and has been shown as a reliable biochemical marker of malignancy. With developments in MR methodology, it is possible to determine the *in vivo* absolute quantification of tCho that provide an opportunity to have cut-off values for the discrimination of normal, benign and malignant breast tissues. Both W–F ratio and the concentration of tCho have been demonstrated to be useful in monitoring the effect of therapy in breast cancer patients. In patients receiving chemotherapy, reduction of W–F ratio and the concentration of tCho were observed in association with the reduction of the primary tumor size indicating its use as non-invasive indicators of favorable clinical outcome of therapy. Studies showed that addition of MRS investigation increases the specificity of MRI. This article reviews some salient features of *in vivo* MRS and its role in the diagnosis and treatment management of breast cancer patients.

**Keywords:** Magnetic resonance spectroscopy (MRS), *in vivo*, localization, breast cancer, water-to-fat (W–F) ratio, choline, biomarker, therapeutic response

## 1. Introduction

Breast cancer is a common disease that affects women and is a major cause of mortality and morbidity [16]. The incidence rate rises with age; the highest being in the developed countries and lowest in Africa and Asia. Breast cancers are highly heterogeneous, and early diagnosis has significant impact on the treatment outcome, survival and on the quality of life of patients. In addition, it is equally important to monitor the tumor response to therapy and recurrence. The traditional detection and screening methods used are mammography and ultrasonography. They significantly increase the patient's survival prospects by facilitating early detection and therapy at an early stage. However, their specificity is low and also has limitations in the diagnosis of lesions in dense breast or microcalcification. Thus, considerable interest is focused on imaging methods that are highly sensitive and specific, relatively non-invasive, cost effective and offers a high predictive value. In this respect, non-invasive techniques such as MR imaging (MRI) and MR spectroscopy (MRS) have been explored as to their potential role in breast cancer [29]. It has been shown that the sensitivity and specificity of MRI for detection of breast cancer can be significantly increased by the use of paramagnetic contrast agents like gadolinium diethylenetriaminopentaacetic acid [29,57]. However, the specificity shows wide range (20–100%) and nearly 50%

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\* Address for correspondence: Prof. N.R. Jagannathan, All India Institute of Medical Sciences, New Delhi 110029, India. Tel.: +91 11 2659 3253; Fax: +91 11 2658 8663; E-mail: jagan1954@hotmail.com.

lesions detected by contrast enhanced MRI turned out as benign by histopathology [37]. However, MR mammography and contrast-enhanced MRI methodologies have greatly improved the ability to differentiate malignant from benign breast lesions in high-risk women. Recently, the role of diffusion MRI has also been evaluated in breast cancer for its role in the diagnosis as well as a tool for assessment of tumor response [47].

## 2. *In vivo* MR spectroscopy (MRS)

Nuclear magnetic resonance (NMR) invented in 1946 has found wide applications in various branches of science like physics, chemistry, biology and medicine and its growth has been phenomenal over the years. The technique developed over the years as the principal method for determining the molecular structure and conformation of biomolecules. However, the most familiar application of NMR to the common man and the clinical community is MRI, which is used to produce anatomic images for diagnostic purposes of various pathologies. It has a major impact in the last two decades in understanding and managing various disease processes that affect humans. MRI is non-invasive and provides anatomic images in multiple planes enabling tissue characterization.

In breast examination, routine MRI and contrast enhanced MRI procedures provide information on the anatomy of the tumor, its extent and pathology; however, the specificity of diagnosis of benign disease from malignant is still poor. Tissues contain several biologically important biochemicals that are distributed throughout the human body in addition to the protons of water and fat. Such tissue biochemical information may be useful for specific diagnosis in addition to anatomic details obtained from MR images. Thus, *in vivo* MRS allows non-invasive detection of the molecular composition of tissues, and provides information on the biochemical and the physiological processes of malignant transformation of cells. Further, it is a unique method that provide cellular metabolism of organs from a well-defined region of interest (ROI) or volume element (voxel). *In vivo* MRS can be performed on nuclei including hydrogen ( $^1\text{H}$ ), phosphorus ( $^{31}\text{P}$ ), carbon ( $^{13}\text{C}$ ), lithium ( $^7\text{Li}$ ), sodium ( $^{23}\text{Na}$ ), fluorine ( $^{19}\text{F}$ ), etc. However, due to high natural abundance and sensitivity, most *in vivo* MRS studies on breast used either  $^1\text{H}$  or  $^{31}\text{P}$  nucleus. This article reviews briefly the results of  $^1\text{H}$  MRS used in breast cancer and its applications related to diagnosis, monitoring of therapeutic response, tumor metabolism and future directions.

### 2.1. Localization methods in $^{31}\text{P}$ MRS

The localization of ROI or a particular volume of interest (VOI) is an important issue of *in vivo* MRS. It is necessary to acquire a MR spectrum exclusively from the VOI with optimal sensitivity and without contamination from outside the voxel. The initial *in vivo* MRS studies used surface coil and was positioned close to the surface of the organ of interest to acquire the spectrum. The early applications of breast *in vivo* MRS were carried out with  $^{31}\text{P}$  nucleus using a surface coil. The energy status, phospholipid metabolites, intracellular pH and free cellular magnesium concentration of tissues are obtained from  $^{31}\text{P}$  MRS. The common phosphorous metabolites that are observed in  $^{31}\text{P}$  MRS of breast tissues are phosphocreatine, inorganic phosphate, nucleotide phosphates, phosphomonoesters and phosphodiester. Malignant breast tumors showed high levels of phosphomonoesters and phosphodiester compared to the normal breast tissues [12,32,38,41].  $^{31}\text{P}$  MRS can also be used for monitoring the response of breast tumors to chemotherapy [32,41]. A decrease in phosphomonoesters was reported in patients who responded to therapy, while an increase reflects the disease progression [17,39,56]. The lower MR sensitivity of detecting phosphorous signal and the requirement of special hardware hampered  $^{31}\text{P}$  MRS

use in characterizing tumors routinely. Further since most  $^{31}\text{P}$  MRS investigations used a surface coil, only rough localization can be achieved and lesions deep inside the organ cannot be accessed. Also, this method of localization has several disadvantages like inhomogeneous transverse magnetic field and contamination of signals from extraneous tissues.

## 2.2. Localization methods in $^1\text{H}$ MRS

Due to higher sensitivity compared to  $^{31}\text{P}$  nucleus,  $^1\text{H}$  MRS is routinely used and has the ability to provide biochemical information from a VOI as small as 1 ml ( $10 \times 10 \times 10 \text{ mm}^3$ ). In addition to water and fat, water-suppressed  $^1\text{H}$  MRS of breast cancer patients showed choline containing compounds (tCho). With the wide availability of 1.5 T MRI scanners, most breast *in vivo* MRS studies were carried out at this field. However, recently, there have been reports on breast MRS at 3 T [13,36], 4 T [7,9,17,34,35] and at 7 T [19,28]. Today, most localization methods use image guidance for placement of the VOI using the images obtained in three orthogonal planes.

For breast  $^1\text{H}$  MRS studies, as in routine MRI examination, the patient is positioned prone in a dedicated single or double breast coil with the breasts fitted into the cup of the coil. To reduce the motion related artifacts, the breast is slightly compressed with cushions. Following localizer images, fat saturated high-resolution images are acquired to identify the full extent of the irregular, spiculated border of malignant tumors. The use of contrast MRI is preferred in most cases; however, in large tumors where it is easy to localize the tumor, contrast may not always be necessary [25]. These images are used for proper positioning of a voxel of an appropriate size, which usually depends on the tumor size. Shimming of the main magnetic field is carried out both globally and over the voxel region to achieve a good magnetic field homogeneity prior to  $^1\text{H}$  MRS. This enhances the probability of detecting a tCho signal in small lesions by increasing the sensitivity. Additionally, a good water and fat suppression further improves the detection of tCho resonance.

Two types of localization schemes are generally used: (i) single-voxel spectroscopy (SVS) and (ii) multi-voxel MRS. Multi-voxel MRS is also referred as chemical-shift imaging (CSI) or MR spectroscopic imaging (MRSI). SVS methods produce signal from a single VOI, while MRSI methods acquire the MR spectrum from multiple voxels simultaneously. Further, using MRSI it is possible to generate metabolite images (metabolic map) in which the pixel intensity is proportional to the relative concentrations of the metabolites and thus provide visual assessment of the spatial variation in metabolite concentrations.

Most breast *in vivo*  $^1\text{H}$  MRS studies have used SVS method centered on the lesion of interest in the breast. The commonly used RF pulse sequences are the double spin echo (PRESS) and the stimulated echo acquisition mode (STEAM) with echo times ranging from 30 to 270 ms. The  $^1\text{H}$  MR spectrum without water and fat suppression provides information on water and fat and the ratio of water-to-fat (W-F ratio) can be calculated [26,50]. The major problem in breast MRS is the detection of resonances from metabolites with low concentrations in the presence of a large water signal and the overlap of the dominant lipid peaks with other metabolites. Thus, water-suppressed and water + fat suppressed spectra are acquired to detect signals due to Cho containing metabolites, which is considered as a biochemical marker of malignancy [10,23,26,27,40,45,48,52,55,59]. The internal water at 4.7 ppm is used as a reference and as a standard in breast MRS. It is important to take account of the age and the menopausal status of the patient during spectral interpretation since post-menopausal women have more adipose tissue compared to glandular tissue in pre-menopausal women.

### 3. Diagnosis of breast cancer using water-fat ratio (W-F) and tCho

The first breast  $^1\text{H}$  MRS was reported using a surface coil [50]. This was followed by few studies using SVS to study the changes in water and lipid content of breast tissues during benign and malignant transformation [23,26,40]. Figure 1 shows the  $^1\text{H}$  MR spectrum obtained without water suppression from a normal breast tissue of a volunteer. The spectrum of a normal breast tissue is dominated by fat peak at 1.33 ppm (methylene  $[-(\text{CH}_2)_n-]$  protons) and the water peak at 4.7 ppm. From the respective peak areas, the water-fat (W-F) ratio can be calculated [23,26,50]. Normal breast tissues are characterized by high fat content while the tumor tissue show higher water content [23,26,30,50]. Normal breast parenchyma is heterogeneous and composed of fat and glandular tissues. Its biochemistry is influenced by physiological changes and hormonal variations during the various phases of the menstrual cycle that affect the MR spectral characteristics. Variation of the MR spectral characteristic and the alterations in the W-F ratio of normal breast tissue of volunteers as a function of the histological phases of the menstrual cycle has been studied [49]. Both the spectral characteristics and the W-F value showed considerable variation, depending on the location of the VOI. Thus, it is important to understand the changes in the biochemistry and physiology of the normal breast tissues [15,49,58]. A change in the lipid content was also found to be associated with tumor development and progression [22,53]. Many reports document substantial overlap of W-F values between benign and malignant breast tissues thus, limiting its utility in diagnosis [23,26,31,40].

Figure 2 shows the water + fat suppressed *in vivo*  $^1\text{H}$  MR spectrum acquired from malignant breast lesion of a patient and is characterized by an intense peak at 3.2 ppm corresponding to several Cho containing compounds like phosphocholine (3.21 ppm), glycerophosphocholine (3.28 ppm) and free choline (3.19 ppm) [51]. Studies report that the elevation of tCho in tumor cells is related to the increased synthesis of cellular membranes [42]. A recent *in vitro*  $^1\text{H}$  and  $^{13}\text{C}$  MRS study reported an increase in both the biosynthetic pathway governed by choline kinase and the catabolic pathways governed by specific phospholipase that contribute to the elevated tCho in tumors [18].

The observation of tCho peak is reported to be specific to malignant breast tissues and can be used to differentiate cancerous from benign tissues [10,23,24,26,27,40,45,46,52,55]. A combined analysis

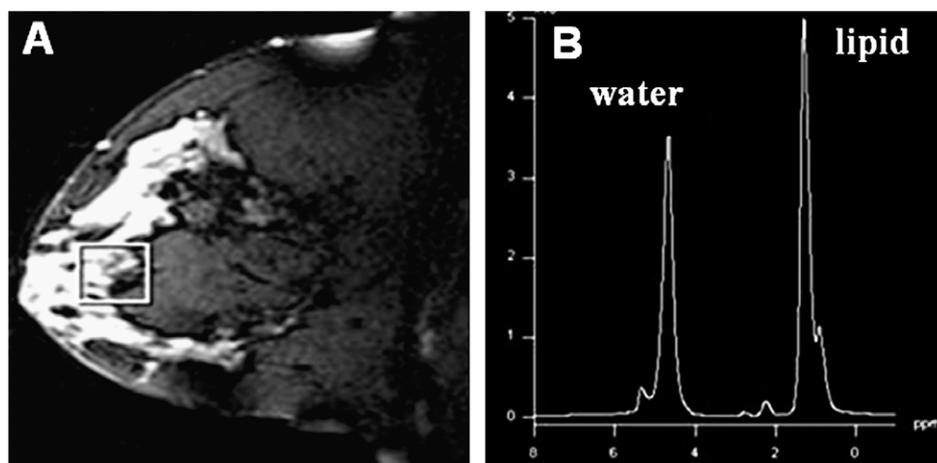


Fig. 1. T2-weighted sagittal image (A) of a normal volunteer showing the voxel from which the  $^1\text{H}$  MR spectrum (B) was obtained without water and lipid suppression.

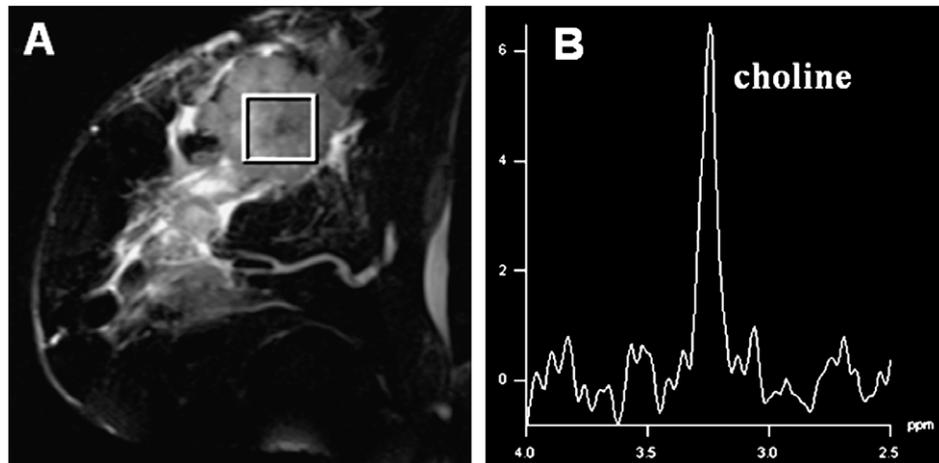


Fig. 2. (A) T2-weighted fat saturation sagittal image of a patient suffering from infiltrating ductal carcinoma of the breast, with the tumor seen as a hypo-intense area. A voxel of  $2 \times 2 \times 2 \text{ cm}^3$  was positioned inside the lesion, from which the  $^1\text{H}$  MR spectrum (B) was obtained with both the water and lipid resonances suppressed. A prominent choline peak is seen at 3.2 ppm.

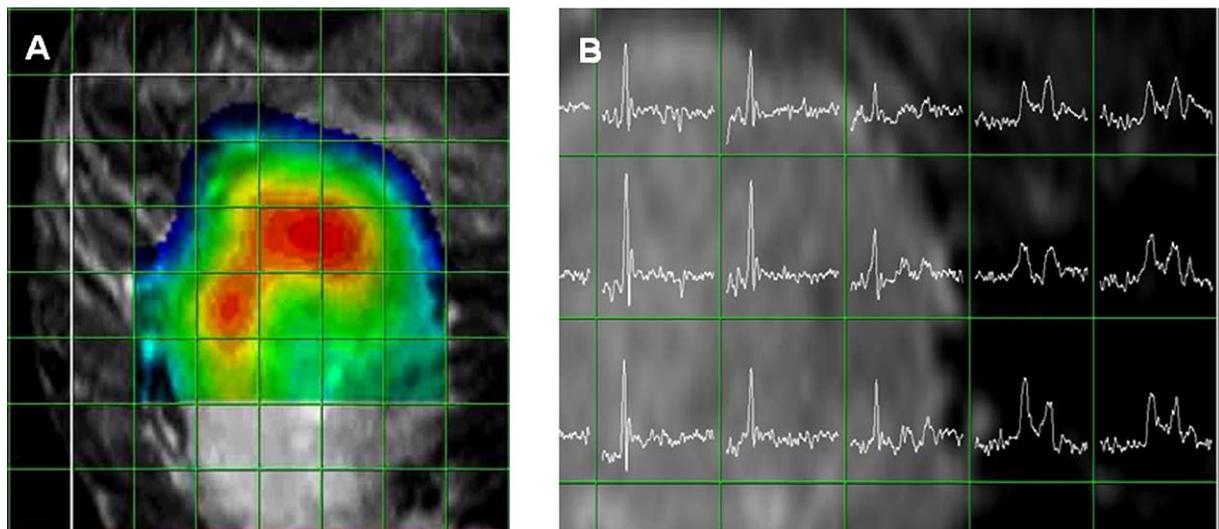


Fig. 3. (A) Axial T2-weighted fat suppressed breast image of an infiltrating ductal carcinoma patient, with the MRSI grid from which multiple voxels are obtained from the tumor region, as shown in (B). (Colors are visible in the online version of the article; <http://dx.doi.org/10.3233/SPE-2011-0522>.)

of the published reports revealed the overall sensitivity and specificity of  $^1\text{H}$  MRS as 83 and 85%, respectively [27]. In younger women, the differentiation of benign from malignant lesions is important, since the incidence of benign breast disease in young women is high. Several studies showed that in younger patients ( $\leq 40$  years of age),  $^1\text{H}$  MRS has a sensitivity of 100% and specificity in the range of 89–100% in detecting malignancy [10,40,59]. Few studies documented the presence of tCho peak in normal breast tissue of lactating women [24,31,52].

Multi-voxel MRS in breast cancer was also found to be valuable in the evaluation of breast cancer tissues [21,22]. Figure 3(A) shows the MRSI grid of a patient suffering from infiltration ductal carcinoma.

The MR spectral pattern obtained from the normal portion of the breast and from the tumor is shown in Fig. 3(B). The advantages of MRSI over SVS include the ability to assess multiple lesions and tissues with normal appearance, as well as to distinguish lesion borders and infiltration into the surrounding tissues [3,11,20–22].

### 3.1. Two-dimensional (2D) in vivo MRS

Single voxel 1D  $^1\text{H}$  MR spectroscopy method is the most commonly used method in breast cancer. However, there is overlap of the dominant lipid peak with other metabolites in 1D MRS and is possible to overcome by the use of localized 2D correlation  $^1\text{H}$  MR spectroscopy methodology. Due to an added dimension, a localized 2D MR spectrum shows better resolution than a conventional 1D MR spectrum. Characterization of invasive ductal carcinoma and healthy fatty breast tissues using 2D MR spectra have been reported [53,54]. Estimation of the relative levels of saturated, unsaturated fatty acids and choline pool are possible using 2D MRS.

### 3.2. Quantification of tCho

The observation of tCho peak in normal, benign and in normal breast tissue of lactating women necessitates accurate quantification of choline rather than using the qualitative assessment of the presence or the absence of tCho resonance for the differentiation of malignant and benign lesions. Two approaches are being followed: (a) semi-quantitative estimation of tCho by calculating the signal-to-noise ratio (SNR), and (b) determination of the absolute concentration of tCho with internal or external referencing.

SNR is measured using the peak height of tCho signal and the noise intensity in an off-resonance region of the spectrum by using the formula  $\text{SNR}_{\text{Cho}} = \text{amplitude of Cho resonance} / \text{RMS amplitude of noise}$ . An SNR value of 1.9 and above was reported to be consistent with malignancy [4–6,44] and can be used to differentiate malignant from benign breast lesions. The use of SNR measured using MRSI method for monitoring the tumor response to therapy also has been reported [11]. However, there are drawbacks in the calculation of SNR due to factors related to the instrument, patient movement, etc.

In comparison to brain, the absolute quantification of tCho in breast is more difficult due to the heterogeneous distribution of the glandular and adipose tissues. Both external referencing and internal water referencing methods have been used to determine the absolute concentration of tCho. The use of water as the internal reference overcomes some of the limitations of the external reference method, like the need for separate calibration experiments and correction for partial volume effects. It also increases the specificity of MRS.

The concentration of tCho from SVS is reported to be in the range of 0.7–2.1 mM using external referencing method [40]. A range of 0.76–21.2 mmol/kg for malignant lesions have been reported using the internal water referencing method [2,35]. Studies from our laboratory [43] showed that the tCho concentration in patients with malignant lesions was  $4.04 \pm 2.08$  mmol/kg compared to benign ( $1.37 \pm 0.83$  mmol/kg) and normal breast tissues ( $0.40 \pm 0.24$  mmol/kg). Choline metabolite quantification at high-field in breast cancer has also been reported [43]. Recently, quantitative multivoxel  $^1\text{H}$  MR spectroscopy in breast lesions also has been reported [14].

## 4. Therapy monitoring by in vivo MRS

*In vivo* MRS has the potential to be used as a non-invasive method to monitor the treatment response in patients receiving therapy [46]. Monitoring the tumor response to therapy is important, especially to

identify non-responders so that appropriate patient management can be initiated. The W–F ratio was reported to be a useful parameter for monitoring the tumor response to therapy in breast cancer patients [26,30,33]. Reduction of W–F value in responders was shown as a non-invasive response indicator of the clinical outcome of neoadjuvant chemotherapy (NACT) in breast cancer patients [30].

The presence of tCho before treatment and its reduction/absence after treatment have been shown as a useful indicator of response. In patients who showed a reduction in tumor size, a significant reduction or absence of the tCho peak after III and/or VI NACT was observed [19,24]. A change in tCho was shown to occur within 24 h of administering chemotherapy that correlated positively to lesion size changes [34]. Recently, our group also reported a significant reduction in the pre-therapy absolute concentration of tCho as early as after I NACT cycle in responders compared to non-responders [46]. Further reduction was observed after II and III NACT cycles. Baek et al. compared the changes in the concentration of tCho and tumor size during NACT between patients who achieved pathologic complete response and those who did not [1].

Like SVS, MRSI method also has been shown to have the potential in the assessment of tumor response of breast cancer patients using ChoSNR [11]. In responders the pre-therapy mean ChoSNR was  $7.8 \pm 5.1$  which reduced to  $3.6 \pm 1.1$  after III NACT in 4 patients while tCho was absent in 10 patients. In non-responders, no statistically significant change in ChoSNR was observed. Similar changes were observed in tumor volume.

## 5. Summary

The growth of *in vivo* MR spectroscopy has been phenomenal in recent years in clinical medicine. This methodology is experiencing rapid expansion and has achieved amazing level of success as an important tool to study several disease processes. Thus in parallel to the MRI developments, the development of  $^1\text{H}$  MRS for characterizing various tumors progressed continuously. The goal of obtaining non-invasive biopsy information through the use of this methodology has pushed the development of several optimized localized MRS procedures with water and fat suppression and editing techniques. *In vivo* MRS can now be used as a unique means to probe the biochemistry of living systems with diagnostic importance by its ability to measure endogenous metabolites non-invasively as well as changes in tissue metabolism. MRS is currently employed for clinical investigation in many sites around the world and presently is acting as a complementary tool to histology, mammogram and other accepted techniques in breast cancer. The ability to perform MRI and MRS non-invasively in the same setting with the same equipment without the injection of radioactive isotopes or blood sampling provides a considerable advantage in patient care. Further, MRS was shown to improve diagnostic accuracy and also as a useful technique to monitor the tumor response to therapy. The addition of *in vivo*  $^1\text{H}$  MRS protocol with the MRI procedure increases the overall acquisition time by approximately 10 min but has the advantage to improve the diagnostic accuracy thus making it a more versatile and widely used technique. Recently, the feasibility of the non-invasive determination of biomarkers of human breast tumor metabolism at 7 T using both  $^1\text{H}$  and  $^{31}\text{P}$  MRI and MRS/I has been reported [28]. Detailed information on the morphology and tissue biochemical information from tumor volumes as small as 10 ml was reported. Such endogenous metabolic information from  $^1\text{H}$  and  $^{31}\text{P}$  nuclei open up the possibility to detect phospholipid metabolites, energy metabolites and pH simultaneously in the human breast *in vivo* and provide a new method for the non-invasive assessment of prognostic and predictive biomarkers in breast cancer treatment.

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