Quantitative Estimate of *in vivo* Metabolites in Breast and Prostate Tissues by MR Spectroscopy

N. R. Jagannathan
Department of NMR & MRI Facility
All India Institute of Medical Sciences
New Delhi -110029, India

1. INTRODUCTION

The diagnosis of cancer is challenging despite the availability of large number of biochemical and imaging investigations. Magnetic resonance imaging (MRI) provides wealth of information on tumor anatomy and physiology. While *in vivo* magnetic resonance spectroscopy (MRS) allow noninvasive detection of molecular composition of tissues and provide information on biochemical and physiological processes associated with tumor progression and regression. Further, quantification of metabolites from a well-defined region of interest (ROI) or volume element (voxel) is possible. The potential of obtaining *in vivo* quantitative biochemical information have revolutionized the field of clinical medicine.

Both MRI and MRS based on same magnetic resonance phenomenon but the technical difference between the methods lies in the use of magnetic field gradients. In MRI, magnetic field gradients are used to achieve spatial information in the form of images. However, in MRS homogeneous magnetic field is necessary to observe the chemical shift differences of biochemical's (metabolites) and therefore no magnetic field gradients are necessary during signal acquisition. The technique of acquisition of MR spectrum from a specific region of interest (ROI) or volume of interest (VOI) of an organ, often referred as "localized *in vivo* MRS", is achieved using MR images. Localization can be single voxel (commonly known as single voxel spectroscopy, SVS) or multi-voxel [commonly referred as chemical shift imaging (CSI) or MR spectroscopic imaging (MRSI)](1,2).

Since the *in vivo* MR spectrum of tissue samples are rich with many biochemicals (metabolites), parameters like estimation of the absolute concentration of metabolites, ratio of intensity of metabolites, etc., provide valuable information (1-3). For example, two important parameters measured from the *in vivo* proton (^{1}H) MR spectrum of malignant breast tissues are: (a) water-to-fat (W-F) ratio, and (ii) tCho concentration (see Figure 1). The basic question

is why do we need quantification of *in vivo* metabolites? The relative levels or concentration of metabolites obtained from *in vivo* MRS provides information on the metabolic abnormalities associated with the disease processes that could be utilized for diagnosis as well as for therapy monitoring. A quantitative method also enables measurements of the magnitude, the rate of tumor response and to compare measurements with potentially different sensitivities as in longitudinal studies where the tumor regresses during therapy monitoring. Further, such measurements provide insight on the metabolic pathway responsible for disease progression or regression.

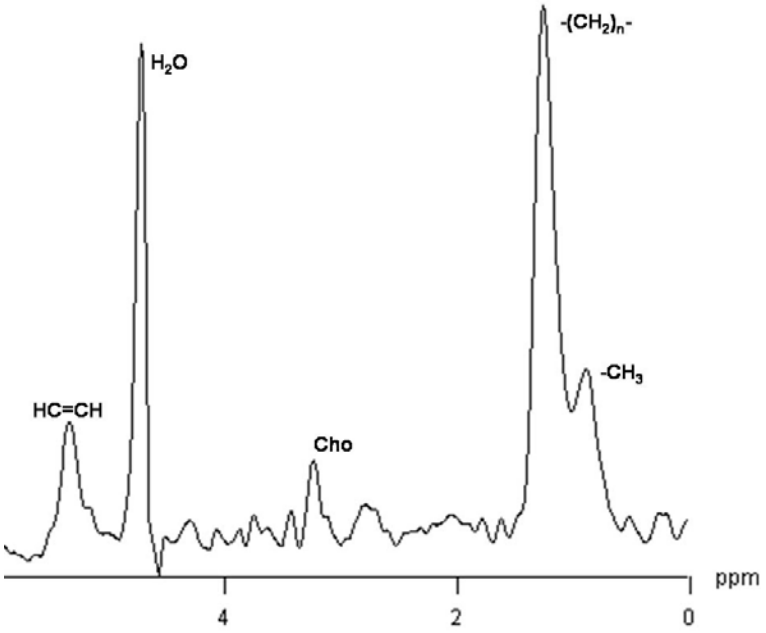

Figure 1: In vivo ^{1}H MR spectrum from a voxel positioned in the tumor of a breast cancer patient obtained with water suppression.

For example, the water suppressed ^{1}H MR spectrum of malignant breast tissues at 1.5T showed a peak at 3.2 ppm corresponding to several choline-containing compounds (tCho) indicative of malignancy. With the advancements in coil technology and with the availability of high-field scanners, report on the observation of tCho has become more frequent in some benign and normal breast tissues also, questioning the diagnostic utility of *in vivo* MRS. In addition, tCho was observed in normal breast tissue of lactating women (2,4,5). These observations have made it essential to develop methods of absolute quantification of the concentration of tCho in malignant, normal and benign breast tissues and to determine the cut-off value for their differentiation.

2. METHODS FOR QUANTIFICATION – DATA ANALYSIS

A method of referencing as well as spectral fitting is required in quantification procedures. Quantitation of MRS data consists of the following steps: (i) to determine the signal intensities (amplitude), (ii) applying corrections factors due to effects of relaxation, scalar coupling, several instrumental and pulse sequence dependent intensity distortions and imperfect localization, and (ii) converting signal amplitude in to concentration.

Model functions and estimation of parameters:

In MRS, the signal amplitude is proportional to the concentration of that metabolite. It is estimated by fitting the spectral data to an appropriate model and evaluating the goodness of fit. This can be carried out either in the time domain (TD) or in the frequency domain (FD). Time domain methods use the measured FID or spin-echo signal, while frequency domain methods process the Fourier spectrum of the original signal. Distortions of the FT spectrum due to imperfections in the measured time domain signal can cause problems in frequency domain analysis methods. This is true for *in vivo* MR signals. Further, the problem areas are spectra with low signal-to-noise ratio (SNR), overlapping resonances and magnetic field inhomogeneity. A FID consists of a sum of damped sinusoids and well-known functions for modeling are $\exp(-\alpha t)$, $\exp(-\beta t^2)$ and $\exp(-\alpha t+\beta t^2)$. The frequency domain functions are Lorentzian, Gaussian and Voigt line shapes. Sometimes complicated damping functions are required for unresolved multiplets.

In quantitation, estimation of model parameters is essential. This is obtained using nonlinear least squares (NLLS) fitting or by Bayesian estimation (BE) and both can be carried out in the time domain or in the frequency domain following FT. Generally, one can restrict the fit to only small subset of the peaks (sinusoids), provided the frequencies in the subset differ considerably from other regions. If the residue of a properly converged fit is above noise, then the function used may not be adequate. In such cases, instead of using Lorentzian shape, Gaussian or Voigt profile may be used. However, problems arise in fitting the water and fat whose model functions are nondescript. In this case, if the baseline is correctly estimated and there is no overlap of peaks, then the peak area can be estimated by integration, without knowledge of the peak shape in the frequency domain. In time domain, the quantitation of nondescript signals is possible and may be represented by a relatively small number of exponentially damped sinusoids. Algorithms based on singular value

decomposition (SVD) are available and these are LPSVD (linear prediction SVD), HSVD (Hankel SVD), HLSVD (Hankel±Lanczos SVD), HD (Hankel diagonalization), EPLPSVD (enhanced procedure LPSVD), and MV-HTLS (minimum variance Hankel total least squares). If the signal is exponentially damped, SVD-based methods are good alternatives to NLLS and the advantage is that the automation is easy. However, prior knowledge other than the number of sinusoids cannot be accommodated, and the S/N below which performance degradation begins (the threshold S/N) is higher than that of NLLS.

In addition, there are number of other fitting programs like jMURI, LC Model, etc. that is used and these provide the concentration of metabolites. Cramer-Rao bounds are used for estimation of error, which however, not suitable at low SNR and one can use parametric method or confidence interval method. The MRUI software is a Matlab/Java based graphical user interface to Fortran based MRS signal processing algorithms such as, AMARES; VARPRO; HSVD; and some other algorithms presently implemented in Matlab. Frequency selective filtering of signals in the MR spectrum is accomplished in the time domain by using the HLSVD (Hankel Lanczos Singular Value Decomposition) algorithm. Once the peaks to be fitted are selected and starting values given and the prior knowledge incorporated into the model function, VARPRO, will try to fit the FID.

In LC Model, deconvolution of spectra is employed using a basis set of reference spectra. The errors in metabolite concentration determined are expressed in percent standard deviation (%SD) of the concentration and represent the 95% confidence interval of the estimated concentration. Further, the concentration of metabolites use Crammer-Rao lower bound and the concentration of metabolites expressed as milli moles per liter (mmol/L).

3. METABOLITE CONCENTRATION IN BREAST CANCER

In brain, quantification of metabolites from *in vivo* MR spectra are routinely performed, however, in breast; it is difficult due to heterogeneous distribution of the glandular and adipose tissues. Haddadin et al. have recently reviewed methods of choline metabolite quantification and the role of high-field MRS in breast cancer (3). Two referencing strategies used for converting measured signal amplitudes into concentrations. As discussed earlier, corrections terms due to effects of relaxation, scalar coupling, several instrumental and pulse sequence dependent intensity distortions and imperfect localization need to be applied to the

data obtained. In the internal water referencing, the water peak from unsuppressed spectra from the same voxel is used as an internal reference. Bolan et al used TDFD method for fitting the breast MR spectra and used internal water referencing for determination of the concentration of tCho at 4T (6). The advantage of TDFD is that it is easy to incorporate any line shape function and frequency-selective properties since the residuals are evaluated and minimized in FD. In general, the use of narrow frequency region is recommended while fitting small resonances like tCho in the presence of huge lipid peaks as in breast MRS.

Further, Bolan et al have worked out the corrected amplitude A of a peak by taking in to account several correction terms related to experimental conditions and the physical properties of each species (6). As per the procedure described by Bolan et al (1), the corrected amplitude A′, is then given by

$$A' = \frac{A}{f_{\text{gain}} f_{\text{coil}} f_{T_1} f_{T_2}}$$

with correction factors:

$$f_{\text{gain}} = gain/gain_0$$

$$f_{\text{coil}} = B_1/B_{1,0}$$

$$f_{T_1} \approx 1 - \exp(-\text{TR}/T_1)$$

$$f_{T_2} = \frac{1}{N} \sum_{j=1}^{N} \exp(-\text{TE}_j/T_2),$$

where, ‘gain’ is the receiver gain, B_1 is the local amplitude of the excitation radiofrequency field, TR is the pulse repetition time, TE is the echo time, f_{gain} receiver correction factor, necessary if receiver gain is different in suppressed and unsuppressed acquisitions. f_{coil} coil receive efficiency factor calculated by assuming the transmit and receive efficiencies equal. $gain_0$ and $B_{1,0}$ (reference values) are arbitrary, but must be used consistently when comparing values from different acquisitions. T_1 correction f_{T1} is approximate (valid provided TR >> TE). With a TE-averaged acquisition, the T_2 correction f_{T2} is a summation over N

acquisitions, each with different TE. Bolan et al. used a constant value for all relaxation constants because it is difficult to measure the relaxation from each voxel (6).

In NMR, the signal amplitudes are proportional to the number of nuclei in the volume. By taking in to account the corrected amplitude, one calculates the ratio of the tCho and water amplitudes, which then is converted to molal concentration (moles solute per mass solvent) by correcting for the number of ^{1}H nuclei per molecule η and the molecular weight of the solvent MW_{water}:

$$[\mathrm{tCho}] = \left(\frac{A}{f_{\mathrm{gain}} f_{T_1} f_{T_2}}\right)_{\mathrm{tCho}} \left(\frac{f_{\mathrm{gain}} f_{T_1} f_{T_2}}{A}\right)_{\mathrm{water}} \frac{\eta_{\mathrm{water}}}{\eta_{\mathrm{tCho}} MW_{\mathrm{water}}}$$

The coil efficiency factor, f_{coil} cancels because both water and tCho come from the same volume of interest. This quantity [tCho], expressed in units mmol/kg, is the metric proposed as an *in vivo* measure of the tissue level of choline-containing compounds in the breast. By knowing T_1 and T_2 of choline and water peak, one can calculate the concentration of tCho peak measured from *in vivo* MRS in breast cancer patients.

By acquiring spectra with maximum receiver gain, Baik et al. (7) used the following equation for tCho estimation:

$$[Cho] = \frac{n_{H_2O}}{n_{Cho} MW_{H_2O}} \times \frac{S_{Cho}}{S_{H_2O}} \times \frac{f_{T_1 H_2O}}{f_{T_1 Cho}} \times \frac{f_{T_2 H_2O}}{f_{T_2 Cho}}$$

and

$$f_{T_1} = 1 - \exp(-TR/T_1) \qquad f_{T_2} = \exp(-TE/T_2)$$

where, S_{Cho}, integral of choline signal; S_{H2O}, integral of internal water signal; nCho, number of contributing ^{1}H nuclei of choline; nH_2O, number of contributing ^{1}H nuclei of water; MWH_2O, molecular weight of water in g/mol; f_{T1} correction factor due to T1 values of choline/water and TR of the sequence; f_{T2} correction factor due to T2 values of choline/water and TE of the sequence. Baik et al. (7) determined T1 and T2 values at 1.5 T and used it: T_1

of Cho = 1500 ms, T_1 of water = 750 ms,T_2 of Cho = 270 ms, and T_2 of water = 97 ms. Substituting these values, the tCho concentration is given by

[Cho]= [(Integral of Cho x 8792.78)/100000] mmol/kg.

In the external referencing scheme, the *in vivo* concentration of tCho is determined and compared to a measurement from an external phantom of known concentration. Bolan et al have worked out that the corrected signal amplitude A′ of a resonance is proportional to the number of nuclei n in the sample and is given by $n = \kappa_{sys}\ A'$ (6). The system constant κ_{sys} (with units' mol/au) accounts for the system specific hardware and software. The value of κ_{sys} is calculated in separate calibration experiments with a phantom of known concentration and measurable relaxation properties. The externally referenced concentration of tCho can then be expressed in molal units (mol/kg) as

$$[\mathrm{tCho}]_{\mathrm{ext}} = \left(\frac{A}{f_{\mathrm{gain}} f_{B_1} f_{T_1} f_{T_2}}\right)_{\mathrm{tCho}} \frac{\kappa_{\mathrm{sys}}}{\eta_{\mathrm{tCho}}} \frac{1}{V \rho_{\mathrm{water}}}$$

where ρ_{water} is the water density, and V is the voxel volume. To compare the internal and external methods, the tissue water density was assumed to be 1 kg/L. The volume V is the entire voxel volume, ignoring the effect of partial volume from adipose tissue (6). The values determined by using the internal and external water referencing methods can then be compared and it was reported that both the methods are highly correlated, although the external method gave somewhat lower values (6).

The advantages of internal referencing is that it is simpler compared to the external referencing method. It automatically corrects for several experimental factors like coil transmit and receive efficiency, voxel size, and compartmentalization with adipose tissue. However, its drawbacks include that it is sensitive to changes in T_2 and water characteristics, which is not the case with the externally referencing method. It is known that breast water content varies under normal physiological conditions and during chemotherapy. This effect can be minimized by accounting for water T2 variations in the quantification method, and by performing externally-referenced quantification of the water content as a control (6).

Some researchers (8-10) have used a semi-quantitative method of estimating tCho by calculating the signal-to-noise ratio (SNR). ChoSNR is measured using the peak height

(intensity) of tCho signal and the intensity of noise from off-resonance region of the MR spectrum using the formula

ChoSNR = amplitude of Cho resonance/RMS amplitude of noise.

Application of *in vivo* quantitation of metabolites in breast cancer studies

(a) Differentiation of malignant, benign and normal breast tissues

The quantification of metabolites opened up many applications in clinical settings, for example, differentiation of malignant, benign and normal breast tissues can be carried out using cutoff values. Initially W-F ratio was used for this purpose and malignant lesions showed high W-F ratio compared to normal breast tissues (11). From the un-suppressed spectra, W-F ratio calculated using the respective peak areas. However, W-F ratio showed significant overlap between malignant with benign lesions thus limiting its usefulness in the differential diagnosis (11). In such cases, determination of absolute concentration will be more useful. Few groups have been working on the method for absolute determination of tCho as per the procedures described above. The reported concentration of tCho in breast cancer patients is in the range of 0.7-2.1 mM using external referencing method (12). Using internal water referencing the concentration of choline was reported to be in the range of 0.76 – 21.2 mmol/kg for malignant lesions (3,6,13). Our group (14) also used internal water referencing method and results showed significantly higher concentration of tCho in patients with malignant lesions (4.04±2.08 mmol/kg) compared to benign (1.37±0.83 mmol/kg) and normal tissues (0.40±0.24 mmol/kg).

Several studies also reported the use of ChoSNR to differentiate malignant from benign lesions and a value of 2 and above was reported to be consistent with malignancy (8-10). A ChoSNR of > 3.2 was reported for diagnosis of malignant lesions using multi-voxel MRS (15). However, the SNR approach has some limitations in that noise is affected by factors related to instrument, patient movement, etc. However, the advantage of multi-voxel technique is that it provides a tool to monitor the changes in ChoSNR simultaneously from the tumor area as well as from normal portion of the breast. Recently, the use of tCho peak integral for differentiation of malignant and non-malignant breast tissues was reported using SV MRS (16).

(b) Monitoring tumor response to therapy

The determination of concentration of *in vivo* metabolites is also useful to monitor the tumor response to therapy to identify responders from non-responders so that appropriate patient management can be initiated. The parameter W-F ratio has been used in monitoring the tumor response to therapy in breast cancer patients (17) with reduction of W-F value observed after therapy in responders. Other parameters like lesion T_2 and tumor volume also have been used in predicting the therapeutic response (18). Further, in patients who showed reduction in tumor size, a significant reduction or absence of tCho peak after III or VI NACT was reported (4). The potential of ChoSNR and tumor volume in the assessment of tumor response by sequential MRSI and conventional MRI was also reported (19). A change in the absolute concentration of tCho was observed within 24 hours of administering chemotherapy that correlated positively to the lesion size changes (13). Work in our laboratory on the tumor response of breast cancer patients using absolute concentration of tCho revealed that changes occur as early as after I NACT in responders compared to non-responders (14). Another group reported changes in the concentration of tCho and tumor size during NACT between patients who achieved pathologic complete response with those who did not using integral values of the tCho peak (20).

4. METHODS OF QUANTIFICATION IN PROSTATE CANCER

The proton MR spectrum (Figure 2) of prostate tissues show metabolites like citrate (Cit), creatine (Cr), choline (Cho), and polyamines (21-23). Cit level is reduced or not detectable in prostate cancer. Most studies until date, report the determination of the absolute concentration of *in vivo* metabolites observed in prostate cancer, except few initial studies (24-26). Liney et al. (26) reported in vivo quantification of citrate in normal and pathological tissues. The mean citrate concentration of tumor in PZ was 5.9 ± 19.5 μmol/g wet weight. This was lower than the mean value observed for normal PZ (42.2 ± 5.8 μmol/g wet weight) and CG (64.5 ± 13.6 μmol/g wet weight).

Generally, the changes in Cit and Cho are quantified by using ratios of metabolites like Cit/Cho, [Cit/(Cho+Cr)] or [(Cho+Cr)/Cit] in most prostate cancer MR studies. The area of Cr peak is added to the area of Cho peak owing to the partial overlap of the Cr and Cho resonances. Moreover, Cr peak remains relatively unchanged in different tissues of the prostate. Since, choline and citrate change in opposite directions, the (Cho+Cr)/Cit ratio emerged as a good quantitative parameter for prostate cancer detection. There have been

several attempts to standardize the interpretation of these metabolic changes [(Cho+Cr)/Cit] using a scoring systems that takes into account not only the ratio but also changes in choline (choline/creatine ratio) and polyamines (22). However, the limitations associated with measuring changes in these metabolites are the inability to accurately quantify changes in these metabolites due to overlap of individual resonances, problems with lipid contamination, and differential T1 saturation effects.

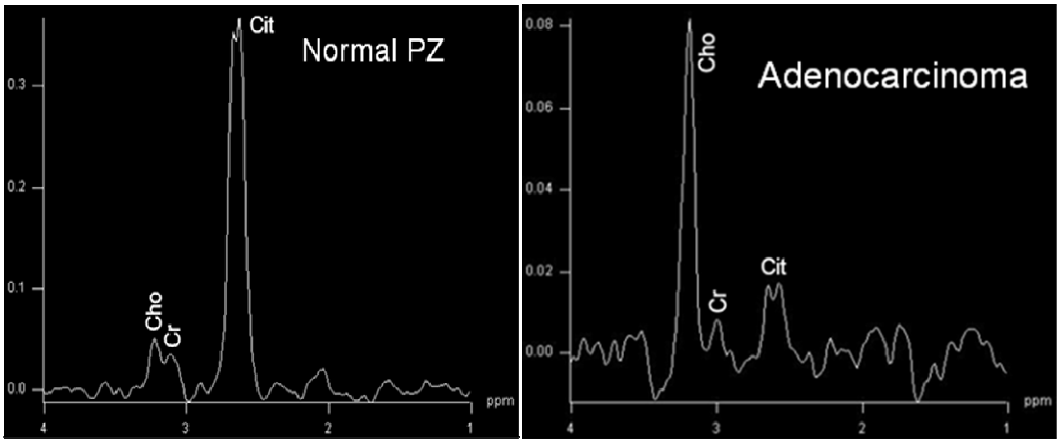

Figure 2: In vivo ^{1}H MR spectrum from the normal PZ of a volunteer (left panel) and the spectrum from the malignant portion of a prostate cancer patient (right panel).

Several researchers used metabolite ratios and showed that ratios that are 2-3 standard deviations (SD) above the mean value can be used to identify cancer (21,22). It was shown that voxels in which the [(Cho+Cr)/Cit] is at least 2 SD above the average ratio may represent possible cancer, while if the ratio is more than 3 SD above the average value, then those voxels are considered to be suggestive of cancer [22]. However, there is no standard cut-off value of the metabolite ratio that can be used to determine the presence of malignancy of the prostate, and there may also be individual variability among patients. From literature one observes that [(Cho+Cr)/Cit] > 0.86 (i.e. 3 SDs above the mean value of the normal peripheral zone, PZ) or [Cit/(Cho+Cr)] < 1.4 (i.e. 2 SDs below the normal mean PZ ratio) have been reported as suspicious of malignancy (22,23). We recently evaluated the potential of [Cit/(Cho+Cr)] ratio in prostate cancer patients prior to biopsy (27). Using ROC method, a cutoff value of 1.2 for [Cit/(Cho+Cr)] ratio was obtained to segregate patients who, upon prostate biopsy, are more likely to show a malignancy in the peripheral zone (PZ) of the prostate gland.

There are several reports that have examined the use of metabolite ratios as a quantitative parameter in the evaluation of prostate cancer (21-23). The values reported by various researchers fall in the same range for cancer patients (23). For example, the Cit/(Cho+Cr) ratio for the normal PZ of patients is found to be in the range 1.46 to 2.16. The value reported by studies from Asia and Europe show broad agreement while that from USA is slightly lower. For the malignant portion of the PZ, the Cit/(Cho+Cr) ratio is between 0.31 and 0.67. Again, the values match from the studies reported from Asia and Europe. The value obtained for BPH cases was in the range 1.21 to 2.01. The (Cho+Cr)/Cit ratio reported by various researchers across the patient population studied fall in the range 0.22 to 0.63 for the normal PZ while cancer PZ shows a wide range from 0.92 to 2.7. However, the spread is less for BPH cases (0.56 to 0.85). A correlation between metabolite ratio and the Gleason grade has also been reported (22). These results indicate the potential of the semi-quantitative parameter (metabolite ratio) in the noninvasive assessment of prostate cancer aggressiveness.

The metabolite ratios has been shown to be useful parameter in the radiation treatment plan to optimize radiation dose selectively to regions of prostate cancer using either intensity modulated radiotherapy (IMRT) or brachytherapy (22); however, this is not being followed in routine clinical practice. Further, the ratios are used to discriminate residual or recurrent prostate cancer from residual benign tissue and atrophic/necrotic tissue after cryosurgery, hormone deprivation therapy, and radiation therapy (22).

SUMMARY

In this presentation, the following were discussed:

a) a brief introduction on the basics of *in vivo* MR spectroscopy,
b) the various biochemical parameters seen *in vivo* MRS of breast and prostate tissues and their significance,
c) methods used for quantitation of *in vivo* MRS metabolites, and
d) some practical tips of how to carry out the absolute concentration determination and their applications related to breast and prostate cancer with examples for:
    i. differentiation of malignant, benign and normal tissues,
    ii. its role in monitoring the tumor response to therapy,

iii. in treatment planning, etc.

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