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TITLE: A Novel Multi-voxel Based Quantitation of Metabolites and Lipids Noninvasively Combined with Diffusion Weighted Imaging in Breast Cancer

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Introduction:

Breast cancer affects one in eight American women during their lifetime and causes more than 40,000 deaths each year (1). Diagnosis and therapeutic management of the breast tumor remain significant medical challenges, hence early detection, diagnosis, and timely treatments are essential to successful health care. High sensitivity is a major advantage of contrast enhanced MRI, but its diagnostic relevance in the future will largely depend on improvements in specificity. Current approaches in the application of MRI to breast tumors aim to improve specificity and sensitivity (2-15). Increased specificity is necessary to reduce the number of biopsies performed to confirm false positive findings. Diffusion-weighted imaging (DWI) is another MR based technique that probes the microstructure of tissues and is sensitive to the degree to which motion of water molecules is restricted in relation to how packed together cells are (16, 17). It has been reported that high resolution DWI may add valuable functional information to conventional MR protocols with short measurement times for the diagnosis of breast cancer and improve the specificity of MR imaging (18-20). However, new technological developments are necessary to assess their role in breast diagnosis. A method capable of identifying biochemical characteristics non-invasively in the tumor lesions that can be used in conjunction with MRI is proton (¹H) MR Spectroscopy (MRS). Researchers have shown that ¹H MRS can be used to characterize breast cancers with improved diagnostic accuracy (21-25). Unfortunately, multi-voxel based novel MR spectroscopic imaging (MRSI) techniques using the speed advantage offered by echo-planar imaging (EPI) and improved spectral resolution offered by two-dimensional (2D) MR spectroscopy (MRS) have not been fully explored in breast cancer studies so far. Hence, this project deals with a combined echo-planar correlated spectroscopic imaging (EP-COSI) and DWI approach for improving the overall specificity of breast cancer detection.

Body:

i) **Proposed Task 1:** To further optimize the multi-voxel based extension of the correlated spectroscopy (COSY) sequence, in which two spectral encodings will be combined with two spatial encodings. This four-dimensional (4D) data acquisition scheme will be accomplished utilizing the echo-planar imaging (EPI) approach that is commonly used for spatial encoding in MRI including DWI. (Months 1-6).

Accomplished during September 2010-May 2011: The proposed 4D EP-COSI sequence was successfully recompiled using the Siemens VB17 platform and the sequence as shown in Fig.1 was implemented on the 3T MRI scanner. The sequence localizes a volume of interest (VOI) using the 90° - 180° - 90° slice-selective radio-frequency (RF) pulses (26).



Figure 1. The 4D EP-COSI sequence to encode two spectral and two spatial dimensions.

The phase-encoding gradient pulses along the G_{phase} enable the encoding along one of the

spatial dimensions (k_y direction). Each of the bipolar gradient pulses along the G_{read} direction was used for encoding the other spatial dimension (k_x). When the bipolar gradient pulses were repeated n times along the same direction facilitated encoding one of the spectral dimensions (t_2). The 2nd spectral encoding was accomplished using the t_1 increment shown in Fig.1. In summary, the implemented sequence enables acquiring a 4D spectral imaging raw signal, s (t_2 , t_1 , k_x , k_y). The Fourier transformation along the encoded 4 dimensions provided two spectral and 2 spatial dimensions.

ii) <u>Proposed Task 2:</u> To evaluate the EP-COSI data using a breast phantom containing two concentric spheres, the inner one containing several metabolites which have been reported in breast tissues surrounded by the outer phantom containing corn oil to mimic fatty tissues known to be in breast tissues, and to optimize the echo speed-factor and other acquisition parameters using the phantom (**Months 1-6**).

Accomplished during September 2010-August 2011: Two different phantoms were prepared, first using various metabolites at physiological concentrations at pH=7.2. The 4D EP-COSI sequence was used to demonstrate the spatial separation of the metabolite and oil phantoms to make sure that the sequence performs properly. The multi-voxel spectra shown in Fig.2 demonstrate that the peaks from the metabolite (left) and lipid (right) phantoms.



Figure 2. *Multi-voxel COSY spectra from a metabolite phantom (left) and a corn oil phantom (right).*

<u>iii) Proposed Task 3:</u> To develop, evaluate and optimize the prior-knowledge basis set spectra using the GAMMA-simulation and breast phantom solutions as prior knowledge for the multi-voxel based COSY spectra recorded using the 3T MRI scanner (Months 3-9).

Accomplished during April 2010-August 2011: The GAMMA library was used to develop different basis-sets for metabolites and lipids in the human breast tissues (27). As shown in Fig.3 (top). the metabolites included three different cholines phosphocholine, (glycerylphosphocholine, gpc; pch; free choline, Ch), phosphoethanolamines (pe) and lactate (lac). The bottom figure of Fig.3 included lipids from saturated fatty acid, mono-unsaturated and poly unsaturated fatty acids.



Figure 3. Prior-knowledge COSY spectra for the breast metabolites (top) and lipids (bottom) developed using the GAMMA library (27).

iv) Proposed Task 4: To record the EP-COSI spectra in the fatty, glandular and ductal areas of healthy breasts. Twenty healthy female volunteers (25-70 years old) with no previous history of breast cancer will be investigated. (*Months 6-24*).

Accomplished during March 2011-July 2011: Five healthy women have been investigated using the MRI protocol with DWI and EP-COSI. The ADC image derived from the DWI data is shown in Fig.4A. Feasibility of recording 2D COSY spectra in multiple regions using the recently implemented EP-COSI sequence is demonstrated in Fig.5B. A metabolite map of the fat peak at 1.3ppm is shown in Fig.4B. 2D MR spectra were recorded in multiple regions including the fatty and glandular regions of the 45 yo healthy subject. A T₁-weighted axial slice MRI is shown on the left side of Fig.4B. The white box represents the VOI localized by three slice-selective radio-frequency (RF)

0 0 0

pulses (90 -180 -90). The total duration for the EP-COSI sequence was approximately 22 minutes. Shown in Fig.5A is an extracted COSY spectrum from one region with a volume of 1x1x2cm. These findings were reproduced in four more healthy women.

Figure 4. **A**) An axial ADC slice image recorded in the same healthy subject using the DWI sequence. **B**) The axial lipid chemical shift image recorded in the 45 yo healthy volunteer using the EP-COSI sequence. Both images are overlaid on top of the T_{1} -weighted MRI.



Figure 5. (A) An extracted 2D COSY spectrum from the EP-COSI data recorded in the 45 yo healthy woman volunteer. In addition to water, presence of 2D diagonal and cross peaks from the methyl, methylene, and olefenic protons of unsaturated and saturated

lipids were only seen confirming the presence of the fatty breast tissue. (B) multi-voxel 2D COSY spectra covering fatty and glandular breast regions (right) of the 45 yo healthy woman volunteer.

Key Research Accomplishments

- The proposed 4D EP-COSI was successfully implemented on the UCLA Radiology Siemens 3T MRI scanner equipped with a dedicated breast phasedarray assembly. This sequence is available now at UCLA only and is not supplied by any of MRI manufacturers. Two phantoms were tested to optimize the sequence performance: the first phantom containing metabolites and the second, containing corn oil.
- The prior-knowledge 2D COSY spectra were developed using the GAMMA library. Previously reported metabolites and lipids to exist in malignant breast cancer and healthy fatty tissues were developed for the prior-knowledge.
- The DWI-MRI protocol combining the 4D EP-COSI sequence was successfully evaluated in a total of 5 healthy women demonstrating the proposed spectroscopic imaging sequence can be combined with clinical breast MRI protocol with the total duration of less than an hour.

Reportable Outcomes:

A. Peer-reviewed Publications: None on Breast Cancer Research based.

B. Presentations: The first abstract summarizing the implementation of the 4D EP-COSI sequence and evaluation of it in healthy women was submitted to the 2011 Era of Hope meeting in March 2011. My participation at the August 2011 Era of Hope meeting in Orlando, FL included two (an oral and a poster) presentations of our work. A 2nd abstract entitled "Novel Multi-dimensional Magnetic Resonance Spectroscopic Imaging: Implementation and Pilot Validation in Prostate and Breast Cancer in vivo" was submitted to the 17th International Biophysics Congress (IUPAB) in June 2011. The invited talk will be presented at the forthcoming conference in Beijing, China (Oct.30-Nov.3, 2011).

<u>C. Books</u>: None on Breast Cancer Research based.

Conclusions: The first 3-4 months of the 1st year was spent in getting the approval from the HSRRB and UCLA IRB offices. The scanning protocol including DWI-MRI and EP-COSI was successfully implemented on the 3T MRI scanner. After optimizing the protocol using two phantoms containing metabolites and corn oil, the protocol was tested in five healthy women. We will continue to recruit 5 healthy women, 10 malignant and 10 benign breast cancer patients during the next year.

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Appendix:

A) Thomas MA, Wilson N, Furuyama J, et al. A Novel Echo-Planar Correlated Spectroscopic Imaging Combined with Diffusion Weighted Imaging in Breast Cancer. Era of Hope conference, Orlando, FL, Aug.2-5, 2011.

B) A copy of our Poster presented at the 2011 Era of Hope meeting.

C) Thomas MA, Furuyama J, Nagarajan R, et al. Novel Multi-dimensional Magnetic Resonance Spectroscopic Imaging: Implementation and Pilot Validation in Prostate and Breast Cancer in vivo. 17th IUPAB conference, Beijing, China, Oct.30-Nov.3, 2011.

A Novel Echo-Planar Correlated Spectroscopic Imaging Combined with Diffusion Weighted Imaging in Breast Cancer

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Background and Objectives: Breast cancer affects one in eight American women during their lifetime and causes more than 40,000 deaths each year. Hence, early detection, diagnosis, and timely treatments are essential to successful health care. Two major goals of this study were: (i) To extend the single-voxel based twodimensional (2D) MR Spectroscopy (MRS) version to multi-voxel based analogue called echo-planar correlated spectroscopic imaging (EP-COSI) for breast cancer. (ii) To record diffusion-weighted imaging (DWI) and to calculate apparent diffusion coefficient (ADC) maps in breast cancer patients and healthy controls. Two hypotheses are being tested: 1) EP-COSI enables full slice coverage of the breast with improved spectral dispersion and sensitivity compared with 1D MRS. 2) Decreased ADC values derived from DWI can be correlated with changing metabolites and lipid levels recorded by the EP-COSI technique.

Methods: The four-dimensional (4D) EP-COSI sequence was optimized using a four-channel breast MRI coil on the Siemens 3T MRI scanner. Several phantom solutions were used for optimizing the sequence performance using the following parameters: TR/TE=1500/30ms, 16x16 spatial encoding using the field of view (FOV) of $16x16cm^2$, 50 t₁ increments for the 2nd spectral encoding and 512 complex points for t₂ acquisition. The DWI images were also recorded in the same setting using the following acquisition parameters: TR/TE=5900/93ms and b= 0, 400 and 800 s/mm2. Both sequences have been tested in healthy volunteers so far.

Results To-date: The ADC maps were calculated using the Siemens ICE platform and Fig.1A shows the ADC slice image recorded in a 45 yo healthy woman. After further optimization, the EP-COSI sequence was tested in the same healthy women. Shown in Fig.1B is the fat image reconstructed from the 4D EP-COSI data.

Conclusions: Our preliminary results so far demonstrate the initial success of two Specific Aims as outlined in the IDEA Expansion grant (W81XWH-10-1-0743). Our immediate future efforts will focus on further demonstrating the clinical potentials of the 4D EPCOSI and DWI sequences in breast cancer patients.



Figure 1. A) An axial ADC slice image recorded in a healthy volunteer using the DWI sequence. B) Axial Fat chemical shift image recorded in the same volunteer using the EP-COSI sequence. Both images are overlaid on top of the T_1 -weighted MRI.

Presented at the Era of Hope Conference, Orlando World Marriott Center, Orlando, FL, August 2-5

P17 - 31 A Novel Echo-Planar Correlated Spectroscopic Imaging Combined with Diffusion Weighted Imaging in Breast Cancer

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Introduction: There are major challenges with accurate diagnosis and therapeutic management of breast cancer, hence early detection, diagnosis and timely treatment are essential to successful health care (1). Diffusion-weighted imaging (DWI) is an RK-based functional imaging technique that probes the microstructure of tissues and is sensitive to the degree to which motion of water molecules is restricted in relation to how packed together cells are (2). Previous research *in vivo* has shown that breast MR spectra exhibit a resonance at ~3.2 ppm that is known to be associated with malignancy (3). A study by Thomas and ocworkers revealed the potential benefit of using a single-vovel (SV)-based two-dimensional (2D) MR Spectroscopy (MRS) sequence in breast cancer to improve detection of information regarding the specific components contributing to the total choline peak *in vivo*(4). Supported by the earlier IDEA grant (#W81XWH-04-1-0656).

Two major goals of this study: (i) To extend the SV-based 2D MR Spectroscopy version to multi-voxel based analogue called echo-planar correlated spectroscopic imaging (EP-COSI) for breast cancer. (ii) To record DWI and to calculate apparent diffusion coefficient (ADC) maps in breast cancer patients and healthy controls.

Two hypotheses are being tested: 1) EP-COSI enables full slice coverage of the breast with improved spectral dispersion and sensitivity compared with 1D MRS. 2) Decreased ADC values derived from DWI can be correlated with changing metabolites and lipid levels recorded by the EP-COSI technique.

Materials and Methods: In contrast with the L-COSY sequence as shown in Fig.1 (Top), the EP-COSI sequence shown in Fig.1 (Bottom) facilitated recording metabolite and lipid levels in multiple regions in a single recording (5). The sequence was recently implemented with the Siemens IDEA VB17 compiler (Siemens Medical Systems, Erlangen, Germany). After initial testing of the performance of EP-COSI using phantom solutions, we have studied five healthy women so far (March-July 2011). The experimental parameters are shown in the middle column. A dedicated breast 4-channel phased array assembly is used for this investigation.





Figure 1: Schematic diagrams of (Top-) the single –voxel based localized correlated spectroscopic (L-COSY),;(Bottom) the echo-planar correlated spectroscopic imaging (EP-COSI).

| naging Parameters | |
|--------------------------|--|
| canner | |
| oil | |
| ubjects | |
| equence optimization | |
| P-COSI voxel size | |
| P-COSI Localizing Pulses | |
| epetition Time (TR) | |
| cho Time (TE) | |
| verages | |
| omplex Points | |
| hase Encoding | |
| 1 spectral width | |
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t1 increments

| | Siemens 3T Trio-Tim (Erlangen, German |
|--|----------------------------------------|
| | Dedicated breast phased array coil |
| | Five healthy volunteers (Mean age 29.3 |
| | Breast Phantom (Corn oil + Metabolites |
| | 1 -2 ml |
| | (90°- 180° -90°) |
| | 1500ms |
| | 30ms |
| | 1 |
| | 512 |
| | 16 |
| | 1190 Hz |
| | 1250 Hz |
| | 50-64 |
| | |

Results and Discussion: A 2D L-COSY spectrum recorded from a 1x1x1cm3 voxel in the lesion of a 55 vo patient diagnosed with invasive carcinoma is shown in Fig.2. The experimental parameters were: TR/TE=2000/30ms, 50 t1 increments for the 2nd spectral encoding, 8 averages per t1 increment and 2048 complex points for t2 acquisition. It took a total of 12-15 minutes to acquire this one 2D L-COSY spectrum. As described earlier (4), there was a significant elevation of water and choline (as covered by a circle in Fig.2), and decline in lipid levels. For comparison, feasibility of recording 2D COSY spectra in multiple regions using the recently implemented EP-COSI sequence is demonstrated in Fig.3B (right). A T-weighted axial slice MRI is shown on the left side of Fig.3. The white box represents the volume of interest (VOI) localized by three slice-selective radio-frequency (RF) pulses (90º-180º-90º). Other sequence parameters were as follows:: TR/TE=1500/30ms, 16x16 spatial encoding using the field of view (FOV) of 16x16cm², 50 t₁ increments for the 2nd spectral encoding, 1 average per t1 increment and 512 complex points for t2 acquisition. The total duration for the EP-COSI sequence was approximately 22 minutes. Each voxel in Fig.3B had a volume of 1x1x2cm3. 2D MR spectra were recorded in multiple regions including the fatty and glandular regions of the 45 yo healthy subject. Shown in Fig.4 is an extracted COSI spectrum from one such region as marked by the arrow These findings were reproduced in four more healthy women. A metabolite map of the fat peak at 1.3ppm is shown in Fig.5A, and the ADC image using DWI is shown in Fig.5B.



Figure 2: A SV-localized 2D L-COSY spectrum recorded in a 55 yo patient with invasive carcinoma



Figure 3: A) The volume of interest (VOI) localized by the EP-COSI sequence (left) and B)multi-voxel 2D COSY spectra covering fatty and glandular breast regions (right) of a 45 yo healthy woman volunteer for the second second



Figure 4: An extracted 2D COSY spectrum from the EP-COSI data shown in Fig.3 recorded in a 45 yo healthy woman volunteer. In addition to water, presence of 2D diagonal and cross peaks from the methyl, methylene, andolefenic protons of unsaturated and saturated lipids were only seen confirming the presence of the fathy breast tissue.





Figure 5: A) Axial Fat chemical shift image recorded in the 45 yo healthy volunteer same as that used for recoding Fig.3using the EP-COSI sequence. B) An axial ADC slice image recorded in the same healthy subject using the DWI sequence. Both images are overlaid on top of the T_1 -weighted MPI

Conclusions: As mentioned in the Specific Aim#1 in our IDEA Expansion grant, successful implementation of the EP-COSI sequence on the 3T MRI scanner to record the multi-voxel 2D MRS in breast cancer was accomplished and five healthy women have been evaluated using this novel 4D MRSI sequence so far. Also, the Specific Aim#3 has been partially completed. Recruitment of benign and malignant breast cancer patients is currently in progress.

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Novel Multi-dimensional Magnetic Resonance Spectroscopic Imaging: Implementation and Pilot Validation in Prostate and Breast Cancer in vivo

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MR spectroscopy (MRS) enables non-invasive detection of several metabolites in human tissues using a whole body magnetic resonance imaging (MRI) scanner. Even though sub-millimeter inplane resolution is achievable using MRI, MR spectroscopic imaging (MRSI) suffers from poor sensitivity (> 1mM) and inferior spatial resolution (>5mm). In spite of these deficits, MRS/MRSI turns out to be a powerful biochemical tool during the past three decades in revealing metabolic abnormalities in cancer, neurological and psychiatric disorders, and other pathologies. Using the enhanced sensitivity offered by the endorectal "receive" technology, researchers have shown changes in choline and citrate levels in malignant compared to benign and healthy prostates where as metabolic images have been recorded with sub-cm resolution. Elevation of choline groups and decline in lipids have been reported in breast cancer using dedicated breast imaging coils. So far, spatial encoding along two or three dimensions has been combined with one spectral dimension only. Hence, there is a necessity for multivoxel based multidimensional MR spectroscopy in a single measurement with clinically acceptable time, and to record spectroscopic information from a large volume of interest subdivided into an array of smaller voxels. By extending the spectroscopic dimensions to two, it has been shown that metabolites which occur at low concentrations can also be detected unambiguously since the resonances from them overlap severely with that of dominant metabolites.

This talk will highlight two major developments: i) Recent progress on the implementation of multi-voxel based two-dimensional (2D) MR spectroscopy on the whole body Siemens 3T MRI scanner combining at least two spatial and two spectral encodings. A second spectral dimension was added to the echo-planar spectroscopic imaging (EPSI), where the first spectral and one of the spatial dimensions were interleaved and phase-encoding gradients facilitated accomplishing the 2nd spatial dimension. Point Resolved Spectroscopy (PRESS)-based localization of volume of interest (VOI) combined with the 2nd spectral encoding added in front of the last refocusing 180⁰ radio-frequency (RF) pulse enabled recording 2D J-resolved spectra from multiple voxels. A slice-selective 90⁰ RF pulse replacing the last 180⁰ RF pulse was used for recording 2D correlated spectroscopy (COSY) in multiple regions. ii) Pilot validation of these novel multi-dimensional MR spectroscopic imaging sequences in prostate and breast cancer using a Siemens 3T MRI scanner will be discussed.

To be presented as an invited talk at the 17th International Biophysics Congress(IUPAB), Beijing, China, Oct.30-Nov.3, 2011.