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NMR SPECTROSCOPY IN MEDICINE: PROMISE, PROGRESS AND PRACTICE

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Over the past fifty years, enormous progress has been made in the resolution and detection sensitivity of NMR. As a result, the use of NMR has spread from physics and chemistry to biochemistry, biology, and finally medicine.

This review covers only a small fraction of the literature on ^{1}H NMR in medicine, focusing on reports with maximum clinical significance, with a bit of history mixed in. We concentrate primarily on the use of NMR in the analysis of breast and prostate cancers, following its evolution from analysis of tissue extracts, to *ex vivo* analysis, and finally to *in vivo* analysis. We also briefly discuss developments such as magic angle spinning, magnetic resonance spectroscopic imaging, and two-dimensional MRS, and improvements in the classification of data.

Key Words: Magnetic Resonance Spectroscopy; Cancer; Prostate; Breast

Introduction

NMR as an analytical tool is now over fifty years old. Enormous progress has been made in resolution and detection sensitivity. This has allowed us to move progressively to study systems of interest in physics, chemistry, biochemistry, biology, and finally medicine. However, success in medical applications came slowly.

Why was the progress slow? Several reasons. There was a cultural gap to cross; medical applications involve complex project approvals; research practices in science and medicine are very different; large numbers of human subjects are required to achieve significant conclusions; data have a wide spread due to the individual characteristics of subjects; new methods for data analysis are required; and detailed consultations between physicians and scientists are difficult to organize due to conflicting schedules. Despite all this, we have made it!

In this review, we shall cover only a small fraction of the literature on ^{1}H NMR in medicine. We shall concentrate on reports that have reached, or have a high potential to reach, clinical significance, with a bit of history mixed in. We shall use the abbreviation MR for general magnetic resonance, with MRI and MRS for the imaging and spectroscopic applications. A more intensive coverage of the field has appeared recently[1].

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MR Methodology

There has been a vast diversity of MR instrumentation developed over the past twenty years. Improvements in the design of superconducting magnets have led to high resolution, vertical bore systems operating at magnetic fields of 21 Tesla (900 MHz for ^{1}H). This has greatly increased detection sensitivity and spectral dispersion for the study of biological fluids, extracts, and tissue specimens. Concomitant with this has been an automation of instrument operation and data analysis, plus the use of multidimensional NMR methods and magic angle spinning.

For the study of human subjects there has been a steady growth in the magnetic fields available and permitted for use. In 1990, 3 and 4 Tesla were considered high fields. Today humans are studied in research mode at fields as high as 9 Tesla. It appears that 3 Tesla will soon be the standard operating field for clinical use. These higher fields offer the usual advantages in sensitivity and dispersion, and also provide enhanced contrast in MRI.

Biological Fluids and Tissue Extracts

The first medical applications of MRS involved ^{1}H studies of human fluids and tissue extracts. A major barrier to the study of fluids was the very strong water resonance – with limited analog-to-digital vertical range, weak resonances could be lost in the noise. A partial solution was drying down of the fluids with redissolution in D_2O. This frequently led to insolubility of proteins,

which only helped to produce spectra of higher resolution. Many interesting results were obtained; for a review see ref. [2]. With present technology fluids may be studied directly in H_2O.

Tissue extracts were often produced using perchloric acid as the extraction solvent. Although the resulting spectra were of high resolution, with interesting differences between patients and controls, their significance was suspect due to the hydrolytic power of the solvent. Proteins, nucleic acids, and lipids could be hydrolyzed by the strongly acidic medium. Hydrolysis products could mask the weak resonances due to low molecular weight metabolites of significance. The alteration of relative intensities of metabolic resonances caused by sample preparation prevented the useful application of multivariate data analysis, the analytical method that has become the gold standard for treatment of medical spectroscopic data.

Despite the caveats above, we would like to present examples of medically useful information obtained from MRS of biological fluids and extracts. There is plenty to be learned by this approach, with appropriate attention to protocol and analysis. In several cases the results obtained from these studies pointed the way for development of the corresponding *in vivo* studies.

MRS of urine has been shown to be useful in determining incipient kidney transplant rejection at subclinical levels. Using optimal region selection software, linear discriminant analysis, and cross-validated classifiers, sensitivity was 93%, and specificity 96%, with a positive predictive value of 96%. MRS analysis of urine for the detection of kidney rejection is simple, fast, inexpensive, and presents less risk to the patient. Post-transplantation monitoring for subclinical inflammation may also make possible earlier, more individualized treatment[3]. Fig. 1 shows data for a patient being monitored after transplant. The y-axis plots the levels of the traditional indicator; when it rises above the indicated threshold anti-inflammatory treatment is applied. The MRS classifier indicated rejection three weeks before the traditional indicator, and during and after the initial treatment. Eighteen weeks after transplant, and after two treatments, the classifier indicated no rejection, which was happily the case.

^{1}H MRS has also been used to analyze the levels of lactic acid in bile. Nishijima *et al.*[4] found lactic acid in the spectra of sixteen patients with hepatobiliary cancers, but were unable to detect it in twelve patients with non-malignant diseases or two healthy volunteers.

Another example of the diagnostic power of MRS of urine is the study by Bamforth *et al.*[5] of patients with inborn errors of metabolism. Using artificial neural nets they were able to distinguish the MR spectra characteristic of seven inborn errors of metabolism. Fig. 2 shows the spectra from three such cases, and

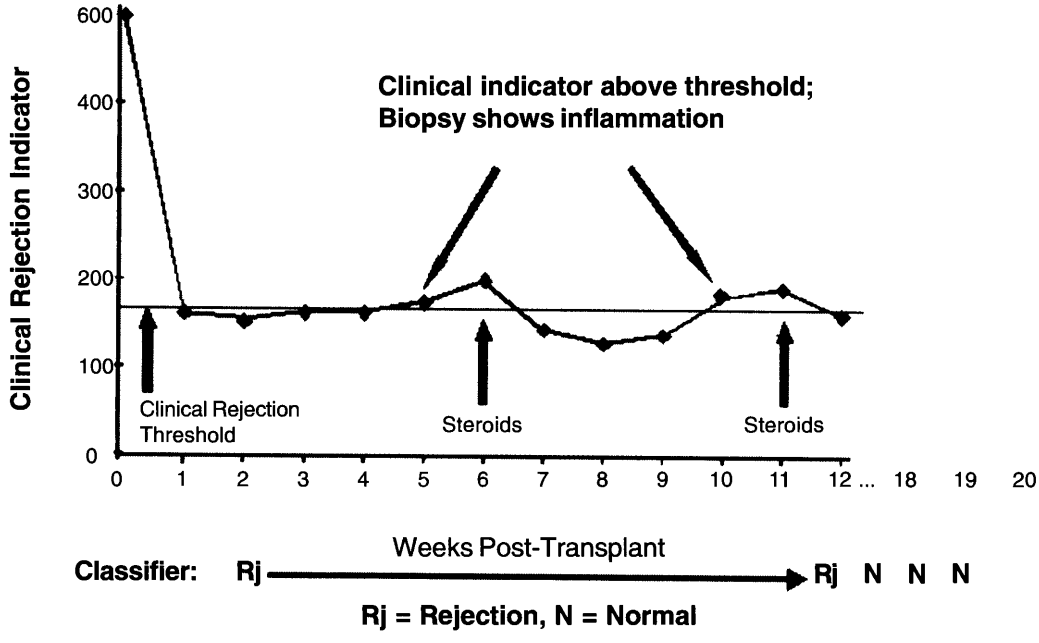

Fig. 1 Post-transplant monitoring of a patient. Anti-inflammatory treatment is given when the indicator rises above the threshold. The MRS classifier indicated rejection three weeks before the traditional indicator[58]. (R L Somorjai, D Rush, R Deslauriers and P Nickerson, unpublished data)

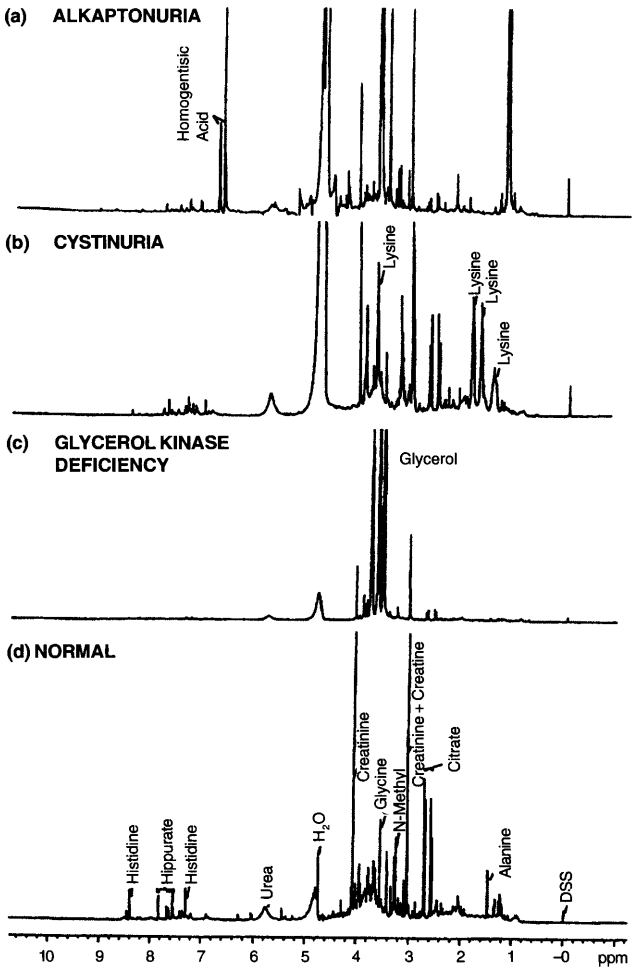

Fig. 2 NMR spectra of urine of patients with inborn errors of metabolism and normal urine at 500 MHz[5].

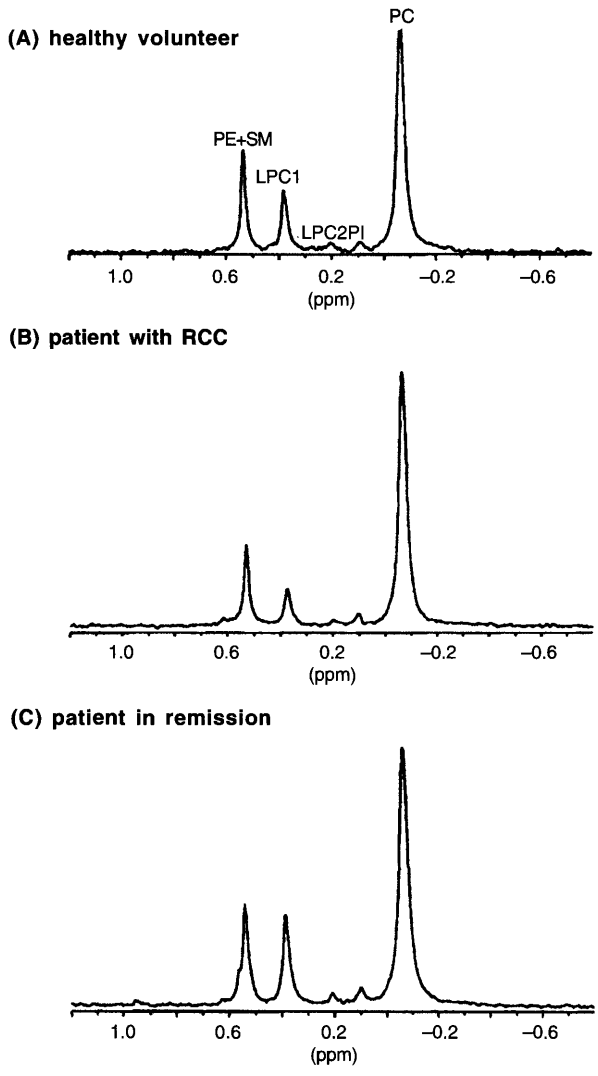

Fig. 3 ^{31}P MR spectra at 7 T of A) a healthy volunteer, B) a patient with renal cell carcinoma, and C) the same patient in remission. Phospholipids are abbreviated as follows: phosphatidylethanolamine plus sphingomyelin, PE + SM; 1- and 2-acyl-lysophosphatidylcholine, LPC1 and LPC2, respectively; phosphatidylinositol, PI; and phosphatidylcholine, PC[8].

that of normal urine. This story also emphasizes the need for advanced spectral analysis methods to separate characteristic distinguishing resonances from the many others found in biological fluids.

^{31}P MRS has value in medical applications given its relatively easy detection and wide separation of resonances. Early studies involved measurement of energy via the intensities of resonances due to ADP, ATP, and CrP. The chemical shift of the inorganic phosphate resonance is a built-in pH meter. More recently attention turned to quantification of lipid classes and the changes in their relative concentration with disease and treatment[6,7]. Fig. 3 shows ^{31}P MR spectra of human plasma from a healthy volunteer, a patient with renal cell carcinoma, and the same patient in remission. The labels on the resonances refer to individual phospholipids. Their relative and absolute concentrations were found to change with the disease, and return towards normal on remission[8].

Tissue MRS

MRS of tissue *ex vivo* is the closest one can come to *in vivo* MRS, but is it relevant? Can it be done with only simple precautions? Are the measurements reproducible? It was long thought that MRS of tissue would yield spectra of broad resonances of little use in diagnosis, due to inhomogeneity of the specimens and immobility of most components in tissue. This turned out to be both right and wrong. As early as 1986, useful data were being obtained on colon tissue at 360 MHz[9]. The very broad resonances due to highly immobilized or high molecular weight species caused little

interference with the narrow resonances due to species of low molecular weight or high mobility.

Sample preparation is critical to obtaining reproducible spectra from tissue samples. The time between removal of the tissue from the subject, and the taking of the MR spectrum must be held constant. Given the difficulty in guaranteeing this, a very practical alternative is immediate freezing of the specimen. With this method, the time between thawing the specimen and taking the spectrum must be controlled. In order to compare spectral intensities from one specimen to another, and to give optimal field shimming, a capillary mounting technique has proven successful[10].

There has been a large number of tissue studies over the past decade, and these have been reviewed recently[11, 12]. Excellent success has been achieved in diagnosis of cancer in brain, thyroid, cervix, esophagus, ovary, colon, breast, and prostate. The latter two will be described below, since they have led to successful applications *in vivo*.

It was for analysis of tissue specimens that the overwhelmingly successful statistical methods for spectral analysis were recently developed. Known generically as Statistical Classification Strategies, they involve a variety of multivariate analysis methods, as well as careful spectral preparation[13, 14].

Breast

In 2000, the estimated number of breast cancer cases worldwide was 1,050,346 and an estimated 372,969 women died of breast cancer[15]. As with most cancers, early detection is key to effective treatment. Current methods of breast cancer screening include palpation (manual breast exam) and mammography. The manual breast exam misses many small, early stage tumors. While mammography may detect some of these smaller tumors, it is limited in its ability to distinguish malignant tumors from benign lesions[16].

Because current screening methods have a low degree of specificity, further examination of breast lesions is required to confirm diagnosis. Ultrasound may be used to evaluate lesions found by breast exam or mammography, but it also has a relatively low specificity. Contrast MRI, while costly, is able to detect breast cancer with a higher level of sensitivity (100%), but only a specificity of 65%[17,18]. Fine needle or open biopsy is often required; biopsies may be guided with the use of mammogram, ultrasound, or MRI[18].

Ex Vivo

Extracts of breast tumour tissue and surrounding normal breast tissue were examined by ^{1}H MRS; the resulting spectra were classified using statistical methods. Large differences in metabolite composition were found between the normal and malignant tissue. Two methods of analysis were used, one analyzing all chemical shifts within the range by neural network analysis (NNA), another using principal component analysis (PCA)[19].

In a very significant study on breast cancer, ^{1}H MRS was performed on fine needle biopsy specimens of benign and malignant breast tissue. Using peak intensity ratios and a limited data set, MRS was able to differentiate between invasive cancer and benign tumours with a reported sensitivity and specificity of 95 and 96%, respectively. Invasive cancer showed an increased signal due to choline-containing metabolites[20]. However, no test of robustness was applied to these data, so it is likely that the real accuracy was appreciably lower than reported. This problem was solved in a second study, where a multivariate (SCS) approach to analysis was employed[21] with both a training set (to develop the classifier) and a test set of data (to test the classifier.) This gave a sensitivity of 92%, a specificity of 93%, and an overall accuracy of 93%. This result is expected to be robust, that is applicable to specimens outside the present cohort. Fig. 4 shows typical spectra of

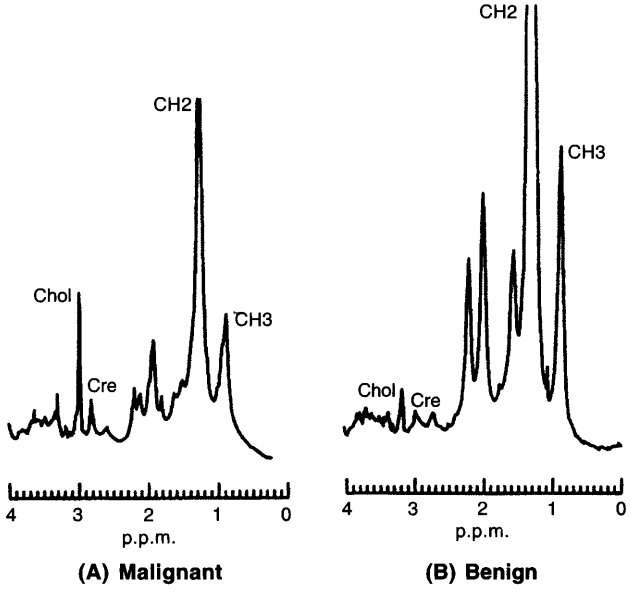

Fig. 4 Typical ^{1}H MR spectra of A) malignant and B) benign tumors at 8.5 T. Abbreviations: choline, Chol; creatine, Cre; methylene, CH2; methyl, CH3, parts per million, ppm[21].

cancerous and normal biopsies. Note the enhanced choline resonance in the former.

A further examination of the above data resulted in a fascinating diagnostic capability. Patients whose breast cancer had spread to distant lymph nodes could be distinguished from those whose cancer was contained, with sensitivity 97%, specificity 96%, and accuracy 95%. Furthermore, the data could distinguish cancers with an established vascular system from those without, with sensitivity 84%, specificity 100%, and accuracy 94%.

This study gives an immediate promise of increased accuracy of diagnosis of breast cancer and its spread, as well as providing valuable information on vascular invasion, leading to improved therapeutic regimens. It is to be hoped that similar diagnostic power can be found in the *in vivo* data, which currently suffer from a paucity of resonances due to the long echo times used for spectral acquisition and decreased spectral dispersion.

HR-MAS

Solid and semisolid specimens usually yield ^{1}H spectra with very broad resonances, due to incompletely averaged anisotropies in chemical shift and dipolar coupling. Spinning the sample at the "magic angle" of 54° with respect to the applied magnetic field, at a rate comparable to or greater than these anisotropies, leads to narrow resonances with excellent resolution. This method is termed high-resolution magic angle spinning (HR-MAS). The specimen must be mounted in a special rotor within a specialized MR probe, and spun at frequencies of 2 –12 KHz. The spectra thus obtained from tissue specimens are impressive in both their resolution and complexity. A difficulty with the method is the large number of resonances that appear in addition to those observed non-spinning, which can swamp the resonances critical to medical diagnosis. This is a relatively new method for MRS diagnosis, and the jury is still out on its effectiveness.

High-resolution magic angle spinning (HR-MAS) ^{1}H MRS has been used to resolve individual resonances within the peaks due to choline-containing compounds. In HR-MAS spectra of healthy, non-fatty breast tissue, choline is prominent and phosphocholine is found only at very low concentrations; however, in ductal carcinomas, phosphocholine is present at higher levels[22]. More research on a larger number of specimens is required to assess the significance of these results.

In Vivo

Gribbestad *et al.*[23] detected breast tumour metabolites *in vivo* using ^{1}H NMR single voxel spectroscopy and a double breast coil. Examination required 45-60 minutes. Normal and neoplastic breast tissue was examined for the presence of choline-containing compounds. Nine of eleven carcinomas and two of eleven benign lesions were found to have choline-containing compounds. Five of seven breastfeeding women also exhibited choline-containing compounds. Analysis of lipid signals was apparently not useful for differentiating breast tissues. Resonances corresponding to choline were also found in spectra of benign breast lesions, including one case of fibrocystic breast disease and one fibroadenoma. Detection of a choline peak in breast by *in vivo* ^{1}H MRS may be a marker of high metabolic activity[24].

Normal and neoplastic breast tissue were examined by *in vivo* ^{1}H MRS and the resulting data was analyzed using artificial neural networks (ANN). With the use of ANN's, benign tumours were differentiated from malignant tissue, but could not be easily differentiated from normal tissue. The use of data from three different echo times in the MRS acquisition sequence improved the success of classification[25]. Fig. 5 shows MRS of a breast tumor. Note the intense choline resonance and

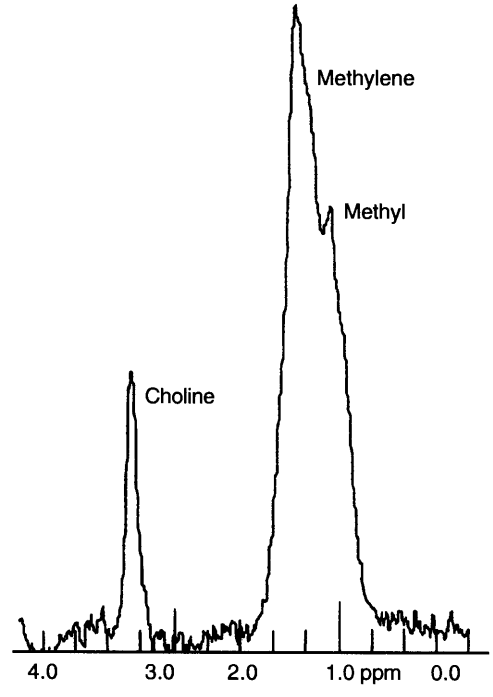

Fig. 5 *In vivo* ^{1}H MR spectrum of an invasive breast carcinoma at 1.5 T. An intense choline peak is seen at 3.2 ppm[24]

the decreased spectral dispersion at the low field of the clinical MRI instrument.

Volume-localized *in vivo* ^{1}H MRS was also used to study the water-to-fat ratios of malignant breast tissue[26,27]. Malignant breast tissue had increased water-to-fat ratios compared to the contralateral unaffected breast and normal breast tissue of volunteers. The water-to-fat ratio showed a statistically significant decrease in patients who had a reduction in primary tumour size as a result of neoadjuvant therapy; reduction in the size of the tumour was confirmed by mammography and clinical examination.

Combination of MRS and MRI

Choline levels were examined in breast lesions by *in vivo* ^{1}H MRS and MRI; elevated levels were found in 19 of 23 breast cancers that were confirmed histopathologically[28]. The specificity and sensitivity of MRS alone were 87 and 83%, respectively, while blinded MRI analysis gave specificity and sensitivity of 86 and 95%, respectively. They suggest that MRS could be used in combination with MRI to improve specificity. Yeung *et al.*[29] also found that *in vivo* ^{1}H MRS can be used in conjunction with MRI to characterize breast lesions. MRS was capable of detecting choline in large contrast enhanced breast lesions with sensitivity 92%, specificity 83%, and overall accuracy 90%. Spectral acquisition required only about 45 minutes and contrast agents had no effect on choline detection.

MRSI

Chemical shift imaging, or MR spectroscopic imaging, which we shall call MRSI, has been shown recently to provide tissue maps in terms of chemical composition, with acquisition times comparable to those used for single voxel spectroscopy. In this method, rather than a single voxel, spectra are obtained over many (10-20) voxels simultaneously. The advantages of this approach are twofold: (1) a large volume of tissue may be interrogated, showing trends from normal to abnormal; (2) the metabolite maps are similar in character to the radiological presentations to which physicians are accustomed. MRSI has been used to examine levels of choline-containing compounds in breast lesions; sensitivity was 78% and specificity was 90%[30] and extensively for the prostate gland, *vide infra*.

2D MRS

Two-dimensional MRS offers the increased resolution of a second dimension. There is a wide variety of 2D pulse sequences[31], but the most widely used in medical applications is COSY. COSY presentations plot chemical shift along both X and Y axes; cross peaks appear where the protons along each axis are scalar coupled.

Two-dimensional correlated MRS was performed *in vivo* on healthy and malignant breast tissue. Results agreed with conventional 1D MRS results, with increased water to fat ratios in cases of cancer compared to normal breast. Surprisingly, 2D peaks due to choline were found in only a few malignant breast tissues. The 2D MR spectra have better resolution than conventional MR spectra making it possible to differentiate peaks due to unsaturated fatty acids from those due to saturated fatty acids[32].

Monitoring/Prognosis

Alterations in the levels of choline in response to neoadjuvant chemotherapy of locally advanced breast cancer were measured by *in vivo* ^{1}H MRS. Before chemotherapy, choline-containing compounds were found in 78% of patients. Following therapy, choline levels were reduced in 89% of patients. Sensitivity of detection of choline-containing compounds in malignant tumours was 80% while specificity was 86%[33].

Yeung *et al.*[34] have measured levels of choline by *in vivo* ^{1}H MRS to evaluate the axillary lymph nodes of breast cancer patients. Compared to fine-needle aspiration biopsy, sensitivity was 82%, specificity 100%, and accuracy 90%; however, when compared to results obtained by surgical lymph node dissection, sensitivity was only 69%, with specificity 100% and accuracy 78%. The latter disagreement may have been due to sampling error in the histopathology. They also found differences in choline levels among different subtypes of cancer. Non-invasive cancers were associated with lower levels of choline-containing compounds.

In all the foregoing *in vivo* studies, it is clear that the low accuracies and conflicting conclusions are due to the small number of subjects studied. Cohorts of fifty subjects or greater are necessary to make the conclusions robust.

Prostate

It is estimated that, in the year 2000, there were 542,990 cases of prostate cancer diagnosed worldwide, with 204,313 deaths due to the disease[15]. Happily, MRS has already become useful in the diagnosis and staging of prostate cancer.

Prostate cancer presents a diagnostic challenge due to the difficulty of differentiating cancer from both normal prostate tissue and benign prostatic hyperplasia (BPH) without the use of invasive techniques. Current methods used for detection and staging of prostate cancer include digital rectal exam (DRE), prostate specific antigen (PSA) measurement in blood, transrectal ultrasound (TRUS), and MRI. DRE is useful for initial screening of prostate cancer, but due to its subjectivity and low sensitivity, it is most useful in combination with other diagnostic methods. PSA measurement is very sensitive; its use in screening has improved markedly the diagnosis of prostate cancer, particularly at early stages. However, PSA testing lacks specificity and many men with benign prostatic hyperplasia (BPH) also have elevated PSA levels. Additionally, PSA is limited by assay variability. Transrectal ultrasound can be used for staging and monitoring of prostate cancer, but is not appropriate for early detection or screening. It is most useful in combination with PSA analysis; alone it can be highly operator-dependent and lacking in sensitivity and specificity for early stage prostate cancer. MRI is most effective for imaging the peripheral zone of the gland. Like the other methods of diagnosis, it is also limited by interobserver variability and an inability to detect microscopic lesions and capsular penetration[35].

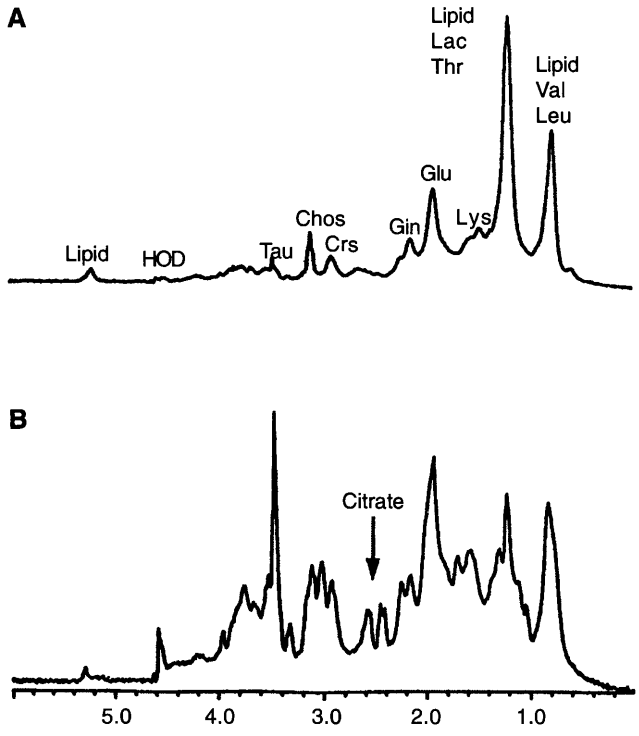

Fig. 6 ^{1}H MR spectra at 360 MHz of A) a cancerous prostate tissue specimen and B) a benign prostatic hyperplasia specimen. The cancerous specimen has increased choline and decreased citrate levels. Abbreviations: partially deuterated water, HOD; taurine, Tau; cholines, Chos; creatines, Crs; glutamine, Gln; glumatic acid, Glu; lysine, lys; lactic acid, Lac; threonine, Thr; valine, Val; leucine, Leu[39].

Ex Vivo

Initial MR studies of prostate were performed on tissue extracts; it became evident very early that the levels of citric acid were indicative of healthy prostate, whereas choline resonances correlated with the presence of malignant disease[36-38].

Hahn *et al.*[39] performed ^{1}H MRS on malignant and benign prostate tissue specimens and subjected the data to multivariate analysis. Analysis was confirmed histopathologically. The overall classification accuracy was 96.6%, with sensitivity 100% and specificity 95.5% in differentiating BPH from prostate cancer. Fig. 6 compares the MR spectra of malignant and benign prostate tissue. Note the decreased citrate and increased choline in the malignant specimen.

^{1}H MRS was also able to distinguish between BPH specimens of glandular or stromal tissue origin; higher levels of citrate were observed in glandular tissue than in stromal[39, 40]. In the latter study histopathology with serial sectioning and MRS were used to characterize prostate pathologies more clearly. It was found that there were two different subtypes of BPH: one (MRS negative) whose spectrum is clearly unlike that of prostate cancer and one subtype (MRS positive) that cannot be easily distinguished from prostate cancer. Prostate cancer was differentiated from normal tissue and BPH by elevated lipid:lysine, lipid:citrate, choline:creatine, and glutamate:lysine ratios. Prostate intraepithelial neoplasia (PIN) spectra were very similar in appearance to those of prostate cancer. McCredie *et al.*[40] also suggest that PIN is a precursor lesion to prostate adenocarcinoma. These data were not submitted to multivariate analysis.

Prostate tissue examined by HR-MAS showed that the measured spermine levels correlate with the volume percentage of normal prostatic epithelial cells determined by histopathology[41]. There was a strong linear correlation between levels of spermine and levels of citrate. Normal tissue exhibited an abundance of spermine and citrate, but cancerous tissue showed reduced levels of both metabolites. The authors suggest that these metabolites are either produced by, or reside in, the normal epithelial cells of the prostate.

In Vivo

Both tissue extracts and biopsy analysis require the taking of a biopsy, which introduces sampling error and causes trauma to the patient. *In vivo* ^{1}H MRS has therefore been attempted with the use of both an endorectal coil (a small surface coil mounted inside a balloon) and pelvic phased-array coils (sets of four surface coils, two located anterior and two posterior to the pelvis)[35]. *In vivo* ^{1}H MRS analysis of the prostate revealed decreased levels of citrate and increased levels of choline in cancerous tissue. The citrate/choline signal ratio was lower in cancerous tissue than normal prostate or BPH tissue; however, due to some overlap of values, this ratio was only useful for the analysis of peripheral zone tumours. Low citrate/choline ratios corresponded to early MR contrast enhancement in tumour tissue. It has also been found that T2-weighted MRI, ^{1}H MRS, and dynamic Gd-enhanced MRI can be combined in one patient examination[42].

In vivo ^{1}H MRS was used to quantify citric acid concentration and water T2 relaxation time of the prostate[43]. Absolute concentrations of citrate in regions of suspected tumour were significantly lower than in normal tissue and BPH. Citrate concentrations in areas of BPH were higher than in the normal peripheral zone; however, there was some overlap in levels[43,44]. Another *in vivo* study has examined severely affected BPH and prostate cancer (PC) patients to establish the most extreme spectroscopic features that differentiate the two conditions. In addition to citrate, creatine, and choline peaks, a peak at 3.6 ppm ascribed to myo-inositol was increased in cases of PC; it was suggested that this may be due to altered membrane metabolism. Advanced PC could be differentiated most clearly from advanced BPH by the ratios of citrate to choline and creatine to myo-inositol peak areas[45]. Decreased levels of the polyamine spermine in the human prostate are associated with prostate cancer. Spermine has been detected in prostate biopsies by 1D and 2D J-resolved ^{1}H MRS. *In vivo,* spermine is difficult to detect due to obscuring resonances from choline and creatine[46]. It is to be hoped that with the improved signal to noise ratios and greater dispersion afforded by high field instruments (3 Tesla or higher), spectra of sufficient quality for high level statistical analysis will be obtained.

MRSI

A variant of MRS that is likely to be more practical for clinicians is MRSI. It can be used to map levels of metabolites over the entire prostate gland. MRSI has been used to assess prostate cancer recurrence in men with high PSA levels who have undergone cryosurgery. Ratios of (choline + creatine)/citrate were used to differentiate among cancer, benign prostate, and normal peripheral zone tissue. MRSI was able to locate all foci of prostate cancer and BPH that were detected by biopsy, and to identify sites that were not detected by biopsy. Due to the destruction of internal prostate architecture by cryosurgery, neither ultrasound nor MRI was able to identify recurrent cancer reliably or to differentiate between viable and necrotic tissue. MRSI appears to be more sensitive than TRUS and biopsy[47]. Fig. 7 compares the MR spectra from cancerous and normal zones of the prostate. Note the intense choline

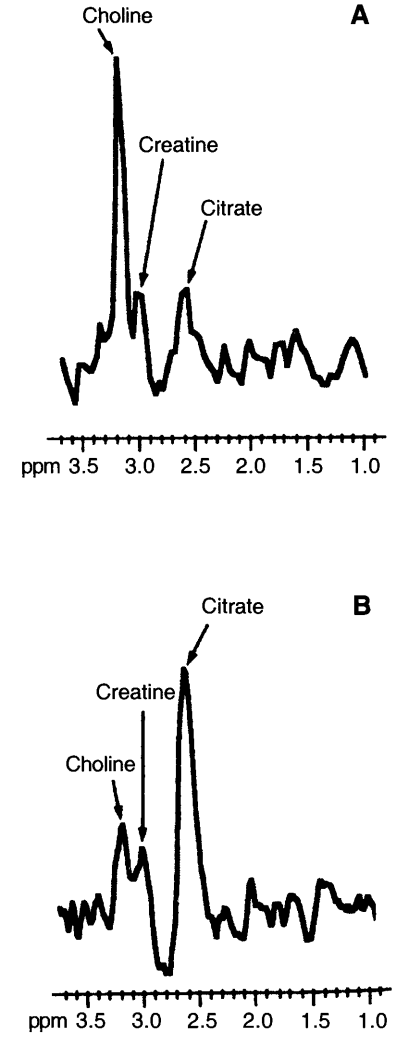

Fig. 7 *In vivo* ^{1}H MR spectra at 1.5 T of A) cancerous prostate and B) normal prostate peripheral zone. The cancerous tissue shows elevated choline and a weak citrate peak[48].

peak and weak citrate peak in the case of cancerous tissue.

MRI and MRSI have been used to localize prostate cancer in patients who had developed post-biopsy hemorrhage. The use of MRSI, in addition to MRI, increased the accuracy of tumour detection from 52% to 75% and the specificity from 26% to 66%, regardless of whether or not the patient had received hormone deprivation therapy[48]. 3D MRSI and MRI were performed on patients who were to undergo radical prostatectomy. The sensitivity and specificity of detection of prostate cancer using a combination of the two methods were greater than those of either method alone[49]. The addition of 3D MRSI to MR imaging improved the accuracy of diagnosis of extracapsular extension in prostate cancer patients and decreased interobserver variability in diagnosis[50]. The accuracy of magnetic resonance spectroscopic imaging was compared to that of MR imaging and biopsy for the intraprostatic localization of cancer. MRSI and MRI were more sensitive than biopsy (76%, 67%, and 50% respectively), but less specific (68%, 69%, 82% respectively). The sensitivity and specificity of MRSI and MRI combined were similar to those of biopsy, except in the prostate apex where the biopsy was not sensitive. It was recommended that the two MR techniques be used to supplement sextant biopsy[51]. The combination of MRSI and MRI has also been found to increase the accuracy of tumor volume measurement in prostate cancer[52].

The transition zone of the prostate is less commonly affected by cancer than the peripheral zone; however this area is more difficult to analyze for the presence of cancer because citrate levels may be variable. As a result, the (choline + creatine)/citrate ratio cannot be used alone. Combined MRI and MRSI have been used to analyze patients for cancer of the prostate transition zone. The two methods combined were able to detect cancer in the transitional zone on the basis of elevated choline levels, a homogeneous dark region on T2-weighted images, and the clustering of suspicious voxels[53].

Monitoring/Prognosis

Accurate monitoring following treatment can help identify patients who need to be treated more aggressively; MRS shows particular promise for the monitoring of prostate cancer. Biopsies taken 18 – 36 months after external beam radiotherapy were examined by ^{1}H MRS, and analyzed by multivariate analysis. Citric acid was invisible in all spectra of post radiation biopsies, regardless of whether they were malignant. Despite the loss of this discriminatory resonance malignant samples were accurately identified by means of the statistical classification strategy with an overall classification accuracy of 91.4%[54].

The metabolic effects of hormone deprivation therapy on prostate cancer patients were studied with the combined use of MRI and 3D MRSI. If the test was performed within four months after the onset of treatment it was as accurate as the initial MR exam. Choline, creatine, citrate, and polyamine levels decreased over time, leading to a total loss of observable metabolites in 25% of patients on long-term therapy. After long-term hormone deprivation treatment the (choline + creatine)/citrate ratio could no longer be used to identify cancer accurately, nor could the presence of elevated choline act as a marker for residual cancer. Levels of all metabolites were lower in healthy tissues than in malignant tissues. Metabolic atrophy may be a useful substitute marker for normal tissue after short-to-intermediate term hormone deprivation therapy; however, as the duration of treatment time increases, metabolic atrophy also takes place in malignant tissue. The absence of citrate and total metabolic atrophy correlated with lower serum levels of PSA. MRSI, in addition to PSA data, may provide useful prognostic information to help assess the efficacy of hormone deprivation therapy[55, 56].

Due to the difficulty of accurately localizing cancer within the prostate gland using current imaging techniques, prostate brachytherapy currently involves placing radiation sources throughout the entire gland. DiBiase *et al.*[57] used MRSI to determine areas of low citrate/(choline + creatine) ratios in prostate cancer, and then selectively targeted these abnormal citrate regions for higher doses of brachytherapy than surrounding areas. MRSI data were successfully incorporated into treatment planning, allowing an increased radiation dose with less risk of side effects. They suggest that MRSI-guided implants are a feasible method of improving prostate therapy.

Classification

More sophisticated methods of data analysis have improved the accuracy of MRS for the diagnosis of

cancer. Traditionally, MRS analysis has involved visual inspection and ratios of peak heights. Increasingly, MRS studies are using statistical classification strategies to analyze data more objectively and to take full advantage of all the data available[13,14,58]. In an exemplary application, multivariate analysis was used on data resulting from ^{1}H MRS performed on malignant and benign prostate tissue specimens. Spectra were divided into training and test sets; optimal subregions were chosen by a region selection algorithm using linear discriminant analysis with the leave-one-out method on the training set. Analysis was confirmed histopathologically. The overall classification accuracy in differentiating BPH from prostate cancer was 96.6%, with a sensitivity of 100% and a specificity of 95.5%[39]. Multivariate analysis has also been used successfully with ^{1}H MRS analysis of post radiation biopsies. Maximally discriminatory subregions were selected using genetic algorithm-driven optimal region selection, cross-validation was performed using a bootstrap method, and linear discriminant analysis was chosen as the ultimate classifier. This method was able to identify accurately malignant samples with an overall accuracy of 91.4% and sensitivity and specificity of identification of 88.9% and 92.0%, respectively[54]. A full description of the statistical classification strategy is given in ref. [13].

Conclusions

Over the past five years there has been a growing acceptance of MRS as a clinical diagnostic. This has varied a great deal from country to country. A critical factor in North America has been the authority to bill for an MRS examination.

The use of MRS for the diagnosis of brain disorders is well documented and is rapidly becoming routine[59]. MRS automation is now available on several commercial platforms. The presentation of spectral data in imaging format has made MRS much more acceptable to radiologists.

Our prediction is that the level of acceptance will continue to grow, and that therefore the accuracy of diagnosis and prognosis of human disease by MR will improve significantly. Subjectivity of conclusion will be largely eliminated.

The challenge now is to make these excellent tools available to a larger number of subjects. As technology has improved and usage of MRI has grown, the standard, lower field instruments have decreased in price. A wide variety of workshops are being held to introduce these methods to radiologists.

The possibility of MRS becoming a routine diagnostic technique is now within sight – a tribute to the collaborations of specialists in physics, electronics, computers and software, and physicians. Congratulations to all.

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