Proton Spectroscopy Provides Accurate Pathology on Biopsy and In Vivo

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In the last 25 years, MR spectroscopy (MRS) has moved from being a basic research tool into routine clinical use. The spectroscopy method reports on those chemicals that are mobile on the MR time scale. Many of these chemicals reflect specific pathological processes but are complicated by the fact that many chemicals change at one time. There are currently two clinical applications for spectroscopy. The first is in the pathology laboratory, where it can be an adjunct to, and in some cases replacement, for difficult pathologies like Barrett’s esophagus and follicular adenoma of the thyroid. The spectroscopy method on a breast biopsy can also report on prognostic indicators, including the potential for spread, from information present in the primary tumor alone. The second application for spectroscopy is in vivo to provide a preoperative diagnosis and this is now achievable for several organs including the prostate. The development of spectroscopy for clinical purposes has relied heavily on the serially-sectioned histopathology to confirm the high accuracy of the method. The combination of in vivo MRI, in vivo MRS, and ex vivo MRS on biopsy samples offers a modality of very high accuracy for preoperative diagnosis and provision of prognostic information for human cancers.

Key Words: spectroscopy; body; pathology; biopsy; in vivo

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PROTON (1H) MAGNETIC RESONANCE SPECTROCOPY (MRS) is now providing an adjunct to and in some cases an alternative to pathology for the diagnosis of human diseases, etiology of pain, and subsequently, pain management. The expectation that MRS would develop at the same rate as MRI produced disappointment and fueled the thought in many that it was an inferior technology.

The reason for the slow emergence of spectroscopy as a diagnostic modality will surprise many. Spectroscopy is highly accurate, providing pathological and in some cases prognostic data with a hitherto unprecedented accuracy (1). So why was the gestation period so long? Spectroscopy was so sensitive it required correlation with very detailed histopathology and patient outcome to prove its accuracy. Unlike MRI, which slotted neatly into the armamentaria of the radiology community, spectroscopy evolved from the collaboration between scientists, surgeons, and pathologists. Only when the groundwork had been done with large and detailed clinical databases on biopsy material did the radiology community become involved.

We have undertaken correlative pathology with spectroscopy on biopsy for 25 years chemically mapping the human body. For the first 15 years, the goal was to develop the technology for analysis of biopsy specimens. Now, with high-field body spectroscopy, some but not all organs can be examined by spectroscopy at 1.5, 3, or 4 T. As with the biopsy program, there is no fast track. If the correlative pathology is not undertaken with appropriate care and precision the outcome will do justice to the sensitivity of the spectroscopy method. A good example of this was an 11% error rate in routine hospital examination of prostate tissue solely due to sampling errors. The errors were identified by MRS and confirmed in a blinded study of serial sectioning of all tissues examined (2).

Neurospectroscopy will not be covered in this review as this was the subject of a recent review by Ross and Danielson (3). This review will also not cover magic angle spinning (MAS) since many of the chemical shifts can be altered due to tissue degradation.

THE EARLY HISTORY OF PROTON MRS

The first report of a high-resolution 1H MRS from intact viable cancer cells was made in the United States by Block (4) in 1973, who suggested the method might lead to pathologically relevant information. In 1978, Daniels et al (5) in Oxford produced the first 1H spectra from rat adrenal glands. A series of experiments undertaken in Sydney in 1978 indicated that MRS could identify the
sequential or stepwise alterations in cells prior to them manifesting frank malignancy, by light microscopy. The examination, by MRS, of excised intact thymus from an inbred strain of mice (6) indicated that the chemical composition of the thymus cells changed prior to such changes being identifiable cytologically or histologically. Thus MRS measured a continuum of changes taking place in the thymus as the final preleukemic period ended and neoplasia occurred (7,8).

Using another mouse model (9), it was shown that 1H MRS distinguished between tumor cells with a capacity to metastasize and those that produced only locally invasive tumors. Assignment of the one-dimensional (1D) MR spectra indicated multiple differences. However, of particular interest was a resonance with a long T2 relaxation rate, at the chemical shift of 1.3 parts per million (ppm), which clearly identified those primary tumors with capacity to metastasize (10–12). The assignment of the potentially diagnostic and prognostic resonances was made possible by two-dimensional (2D) MR methods (13–15). The majority of the resonance assignments were made by 2D MRS and many correlated with biological or clinical criteria.

It is now known that these initial observations recorded on animal models could be extrapolated to cells, biopsies, and tissues in vivo (1). Importantly the capacity of MRS to determine both neoplastic status and biological potential from human biopsies with histological correlation approaching 100% has now been demonstrated (16).

**HISTOPATHOLOGY**

Histopathology has been the gold standard of the 20th century but it is also well recognized that correlation with clinical outcome is one of statistical probability rather than a medical certainty. In the first instance MRS was tested for its capacity to discriminate between well documented and highly reproducible stages of pathological process such as cancer and precancer of the uterine cervix (17). Later, MRS was used to determine if there were useful spectral differences between histological variants within a neoplastic range that correlated closely with predicted clinical outcome. The question being addressed was “could MRS do what pathologists found difficult or not possible.” Two good examples are follicular neoplasms of the thyroid (18) and dysplasia in Barrett’s esophagus (19).

Much of the earlier difficulties stemmed from extreme sensitivity of the MRS method and the need to examine with great care the histological features of the test sample taken from a lesion, the nature of which varied from area to area. It was not possible to simply to use the crude “global” pathological diagnosis of the patient’s salient disease as a measure of the expected MR findings and hope that the correlation would be other than mediocre. For the MRS method to achieve its full potential, detailed step-sectioning of the specimen for histopathological analysis of the precise piece of tissue examined was needed. The exact percentage of each type of tissue type or cellular composition needed to be recorded. For example, for the prostate percentages of glandular, stromal, prostate intraepithelial neoplasia (PIN), and inflammatory disease need to be compared with the amount of malignant tissue present in each slide. These meant large volumes of pathology, clinical, and spectral information were compiled for evaluation. This principle applies today to examination of the resected organ following in vivo spectroscopy.

**THE BASIS OF THE MRS METHOD**

MRS reports on pools of chemicals in cells and tissues as they alter (many at the same time) with aging and the development of disease processes (17,20–40). The molecules in question may be metabolites or originate from areas on the cell that also allow molecular motion such as cell-surface markers or mobile lipid pools. A summary of assignments made for both 1D and 2D spectroscopy of cell and tissues are listed in Tables 1 and 2.

Many of the biochemical processes that are amenable to being monitored by MRS occur in parallel, but the chemical manifestations do not necessarily alter in the same direction; i.e., some can be commencing while others are shutting down. In a 1D spectrum there are hundreds of resonances forming composites with different T1 and T2 relaxation values. With time it can be determined which of the chemical criteria for each organ and disease state are important and which have the capacity to interfere with a definitive diagnosis.

**SHIMMING**

Optimization of magnetic field homogeneity (shimming) is crucially important for spectroscopy. The first experiments undertaken on cells and tissues preceded automatic computer based shimming (41,42). The latest generations of vertical spectrometers have pulse field gradients available allowing gradient shimming. What used to be considered an “art” was transformed into an automated operation.

Body spectroscopy in vivo still suffers from lack of adequate automated shimming particularly at the higher field strengths of 3 T and above. It is particularly important for in vivo spectroscopy to have the higher order shims available. The intricacies of shimming are well covered in a two part series by Dr. W. Hull (41,42). For body spectroscopy to reach its full potential this is an area in need of attention. However the problem is no worse than trying to shim on biopsy specimens in the late 1970s!

**SPECTRAL EDITING**

The MRS method is amenable to two types of spectral editing, during data acquisition and postacquisition (15). The advantage to postacquisition processing is that, in principle, no resonances are lost due to filtering and are available for a series of different processing protocols. The many assignments can be made by 1D and 2D spectroscopy (43). By comparing resonance intensity and, in some cases, line width at half height (a dangerous option in the view of these authors) correlations with clinical criteria can be made.
EFFECTIVE CONTRAST MATERIAL ON SPECTROSCOPY

Whether or not contrast material may be administered before clinical spectroscopy appears to be organ-dependent and warrants consideration (44).

STATISTICAL CLASSIFICATION STRATEGY—MANAGING LARGE VOLUMES OF DATA

The interpretation and management of complex databases including large volumes of biomedical data including spectroscopy, pathology and patient statistics, requires a special methodology. Pattern recognition methods are now in common use for many biophysical applications including proteomics and metabolomics (45).

A multistage statistical classification strategy (SCS) was developed specifically to address these issues. The method has allowed automated analysis of both ex vivo and in vivo spectroscopic data using highly accurate and reliable mathematical classifiers for a variety of clinical applications. For reviews see Lean et al (16) and Somorjai et al (45). The application of SCS methodology provides classifiers that distinguish, for example, benign from malignant specimens, with accuracies approaching 100% when a small proportion of samples that are unable to be accurately classified are excluded (often due to lack of signal-to-noise ratio [SNR]). When these samples are included, accuracy percentages in the mid-90s can still be achieved.

Unlike most other mathematical methods of data analysis the SCS method identifies the spectral regions from which the diagnostic and prognostic information is taken. This allows the chemical resonating in these regions to be considered and the biochemical pathways involved in the disease process analyzed. However, it must be remembered that with such a method the results are only as good as the pathology included in the database and that adequate numbers of samples must be included in the training sets to ensure that the data is not overclassified.

BREAST

Breast cancer is the most common cancer to affect women in Western countries, with an incidence of about 80–100 newly diagnosed patients per 100,000 inhabitants per year (46). Presurgical assessment of the breast lesion with accurate sizing spatial location and an accurate assessment of extent of disease has the potential to radically improve patient management.

Comparison of Breast Diseases on Fine-Needle Biopsy at 8.5 T

The 1H MR spectra obtained from breast fine-needle aspiration samples that were taken from more than...
1000 breast lesions during surgery or through skin preoperatively (Fig. 1) showed a clear distinction between frankly malignant, ductal carcinoma in situ (DCIS) with microinvasion, DCIS without microinvasion, and benign tissue (47,48). The correlative histopathology (shown on the right in Fig. 1) was obtained by the surgeon intraoperatively by taking a tissue sample from the end of the needle track to ensure the spectra were accurately compared with the correct diagnosis. It is clearly seen in Fig. 1 that an increase in cellular metabolites that are monitored by MRS occurs during tumor development. Of particular importance is the appearance of a large number of chemically active species that are present in the spectrum from DCIS with microinvasion and not apparent in the spectrum from a specimen of DCIS without microinvasion. It is further stressed that special techniques such as immunohistochemistry may be needed to discriminate between closely related stages in a neoplastic spectra such as the presence or absence of microinvasion in a cancerized lobule associated with DCIS (Fig. 1b and c). However, on visual inspection of the MRS data, fibroadenoma remained a false positive (47).

It can also be seen in Fig. 1 that there is a clear distinction between the spectra from frankly malignant tissue and DCIS with microinvasion and those from benign and DCIS without microinvasion. Differences include increased choline to creatine ratios and increased in the amounts of chemicals in 2.0 –2.4 ppm spectral regions with the capacity for invasion. These spectral differences are consistent with the literature reports showing that choline metabolites alter with aging and cancer in the breast. MRS of breast cell lines showed that the total choline-containing phospholipids metabolite levels increase with progression from normal to immortalized, to oncogene-transformed, to tumor-derived cells (35). Aboagye and Bhujwalla (35) demonstrated a glycerophosphocholine (GPC) to phosphocholine (PC) switch with cell immortalization in oncogene-transformed and breast tumor cell. They also reported an increase in PC levels and total choline-containing phospholipid metabolite levels. Sitter et al (49) used MAS MRS to analyze breast tissues and perchloric acid extracts and assigned more than 30 metabolites. However, the chemical shifts recorded for MAS and for cell extracts are not necessarily the same as

### Table 2
Assignment of Resonances in 1H MR COSY

<table>
<thead>
<tr>
<th>Molecules</th>
<th>Abbreviation</th>
<th>Moiety</th>
<th>Chemical shift (ppm)</th>
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<tr>
<td>Lipids</td>
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<td></td>
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<tr>
<td>Triglyceride</td>
<td>A</td>
<td>(CH₃)₂-C₂H₅-C₂H₅</td>
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<tr>
<td></td>
<td>B</td>
<td>=C=CH₂-C₂H₅</td>
<td>1.33 2.06</td>
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<tr>
<td></td>
<td>C</td>
<td>-CH=CH₂=C=CH₂</td>
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</tr>
<tr>
<td></td>
<td>D</td>
<td>-CH₂=CH=CH₂</td>
<td>2.84 5.38</td>
</tr>
<tr>
<td></td>
<td>E</td>
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<td>1.33 1.62</td>
</tr>
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<td></td>
<td>F</td>
<td>O=C-C₂H₅-C₂H₅</td>
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<tr>
<td></td>
<td>G</td>
<td>-O-C₂H₅-C₂H₅-O</td>
<td>4.12 5.26</td>
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<tr>
<td></td>
<td>G'</td>
<td>-O-C₂H₅</td>
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<td>Amines</td>
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<td>Chol</td>
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<td>Phosphoryl-choline</td>
<td>PC</td>
<td>N-C₂H₅-C₂H₅-OP</td>
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<tr>
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<td>GPC</td>
<td>N-C₂H₅-C₂H₅-OPO</td>
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<tr>
<td>Amino acids</td>
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<td>3.60 3.90</td>
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Clinical Study of Fine-Needle Aspiration Biopsy Specimens at 8.5 T

One of the most revealing studies into the diagnostic power of $^1$H MRS analyzed by the SCS method is the recent study of fine-needle aspirate biopsies (FNAB). The quality of spectra obtained from these specimens can be seen in Fig. 1 and assignments in Table 1. Visual inspection of these spectra where the choline to creatine ratio was measured gave an accuracy of 96% for determining malignant status. Using the SCS method the distinction between benign and malignant biopsy specimens gave a sensitivity of 95% and specificity of 96% with an overall accuracy of 96% (Table 3). Interestingly, fibroadenoma ceased to be a false positive when the SCS method was implemented to analyze the MRS data. The SCS method did not improve on malignant vs. benign but the rest of the information available is not obtainable by another single method to date.

From the same spectra obtained from tissue in the primary tumor and using the SCS method, lymph node involvement could also be predicted with an overall accuracy of 95%. Similarly, from the same spectrum from the primary tumor, vascular invasion in the definitive surgical specimen was predicted with an overall accuracy of 94% (47) (Table 3). Estrogen and progesterone receptor status was correctly predicted with 87% and 91% accuracy, respectively (31), and low grade (Grades 1 and 2) distinguished from high grade (Grade 3) tumors with an accuracy of 94.7%. This is the first clinical report of information on the invasive, metastatic, and receptor status of a tumor being available by inspection of a cell sample of the primary tumor.

The MRS method is dependent upon there being sufficient cells for an adequate SNR in the spectrum, i.e., $5 \times 10^6$. A routine audit of FNAB cytology specimens showed there was a 15% rate of inadequate cellularity in the specimens (50). Also, it should be noted that the MRS method is not currently robust when applied to core biopsies due to the high content of fat. The effect of high levels of fat on an MR spectrum is described below.

Thus with adequate sampling the application of MRS to the examination of FNAB from the breast has the potential to revolutionize breast cancer management by providing both diagnosis and staging parameters prior to surgery. The number of patients examined so far is just over 1000. It is anticipated that a further 1000 will be needed for robust classifiers to be available. The method is currently undergoing clinical acceptance testing.

**Spectroscopy of The Breast In Vivo at 1.5 T and Higher Field Strengths**

There are now a series of reports on the application of MRS to breast in vivo. A range of breast coils and pulse sequences have been utilized. It was initially, and continued to be, proposed that the broad composite resonance from choline and choline containing compounds at 3.2 ppm was a marker for malignant disease (51). This observation has been contentious for many years, bringing into doubt whether MRS could be diagnostic for breast cancer in vivo (51–58). At the higher frequency of 4 T, the intensity of the choline resonance relative to other resonances was also considered as a possible diagnostic method (59).

A flaw in these studies (except for Kvistad et al (54)) was the absence of a cohort of apparently healthy volunteers and lactating mothers. The term "broad composite resonance" gives license to a range of chemical shifts and with such a rudimentary means of visual inspection of the data some of the volunteers, and all of the lactating mothers, had a broad composite resonance in roughly the 3.2 ppm spectral region.

Stanwell et al (60) demonstrated that, with careful referencing, the spectra from women with invasive malignant disease had a resonance at 3.23 ppm, whereas in the spectra obtained from those women that had healthy breast tissue or lactating mothers (Fig. 2), the chemical shift was different, at 3.28 ppm. With this discrepancy corrected the sensitivity and specificity of the method was 84% and 100%, respectively, leaving a 16% false-negative of concern. This can be explained by the basic chemistry of the breast.

As was initially described by Roebuck et al (53) the variable and intense contribution to the MR spectra from lipid and fat in the breast poses a problem for 1D spectroscopy. In the Stanwell et al (60) study, it would...
Increasing levels of chemicals with a short $T_2$ will mask smaller amounts of chemicals with a long $T_2$.

**Figure 3.** Schematic representation of the effect of increasing levels of lipid (short $T_2$) on those resonances with a long $T_2$ relaxation value, e.g., choline (C.E. Mountford, unpublished results).

appear that in approximately 84% of patients with malignant disease the choline resonance at 3.23 ppm can be seen despite the signals from fat. In the remaining 16% the fat is superimposed over choline-containing compounds, thus masking the diagnostic markers.

The effect of increasing levels of lipid, short $T_2$ relaxation value, on smaller amounts of chemicals (choline-containing metabolites, with a long $T_2$) is shown in Fig. 3. The $T_2$ relaxation value and hence the slope does not alter, but with increasing amounts of lipid the resonances with a long $T_2$ are masked. Lipid suppression techniques cannot be guaranteed to overcome this problem; hence, if choline containing metabolites are not observed, it cannot be assumed that they are not there. The potential for false negatives can only be overcome by the implementation of 2D correlated spectroscopy (14,61), which will separate the lipid from the metabolites in a second dimension. This technology has now being extended to magnetic resonance spectroscopic imaging (MRSI), providing the ability to monitor multiple lesions in the breast (62).

Now and in the immediate future the combination of contrast enhanced MRI and in vivo MRS to identify the lesion(s) and an image guided biopsy that is subsequently examined by MRS at 8.5 T offers a diagnostic procedure likely to result in an extremely high level of diagnostic accuracy.

**PROSTATE**

Prostate carcinoma is the most common cancer affecting men in the United States (63) and Australia (64). Histological examination remains the standard for diagnosis and classification of prostate neoplasms (65), yet correlation between histological features and clinical outcome is modest at best. Prostate cancers demonstrate a range of biological potential. The options facing the patient are clinical observation, hormone deprivation therapy, surgical procedures, and radiation or cryosurgical therapies. Disease in the prostate is heterogeneous and, therefore, spectroscopic mapping of the prostate offers an opportune means of accurately identifying spatial location and diagnosis.

In contrast to all other organs, except perhaps the brain, the first example of spectroscopy of the prostate was undertaken in vivo in the late 1980s using an endorectal coil (66–68). Subsequently the University College, San Francisco (UCSF) group, led by John Kurhanewicz, S.J. Nelson, and colleagues, has developed in vivo spectroscopy of the prostate such that it is now implemented on a routine basis in approximately 40 hospitals in the United States. In Europe, Heerschap et al (69) and van der Graff et al (70) have developed a similar method, albeit with software developed for a different manufacturer. Both use an endorectal coil, allowing relatively high-resolution anatomical images as well as 3D chemical shift imaging (CSI) (71).

The in vivo diagnostic method is based on high levels of citrate that are present in healthy tissue. In contrast, malignant tissue is characterized by low levels of citrate and increased levels of compounds involved in phosphatidyl choline and phosphatidylethanolamine synthesis and hydrolysis (choline, phosphocholine, glycerophosphocholine, ethanolamine, and phosphoethanolamine) contributing to the spectral region known to contain choline.

The development of in vivo MRSI (see Kurhanewicz et al (72)) was a major advancement providing a 3D map of contiguous volumes of 0.24–0.34 cm$^3$ of voxels that map the entire prostate (Fig. 4). The MRS and MRSI are acquirable within the same examination and alignment is possible. This can be seen in Fig. 4d. The citrate resonance is absent but there is a strong choline resonance apparent. In contrast, in Fig. 4e there is a strong citrate resonance but choline and creatine are also present at significantly smaller than a citrate resonance. The in vivo method involves suppressing the lipid both inside the voxel of interest and outside the voxel of interest.

Initial results of endorectal 3 T $^1$H MR spectroscopic imaging showed spatial, temporal, and spectral resolution advantages at the higher field strength (73). The comparison of the 1.5 and 3 T data is seen in Fig. 5. The spectroscopic data collected at 3 T show the relevant metabolites to be separated from each other and from water and lipid, improving the diagnostic capability. However, this study, like most at 3 T, indicates that shimming at 3 T is considerably more difficult.

It was almost a decade after the first in vivo study of the prostate that the first comprehensive study on prostate biopsies was published by the National Research Council of Canada at the higher frequency of 8.5 T (40). Using the SCS method, the sensitivity and specificity achieved in this study were 100% and 95% for distinguishing benign prostate hyperplasia (BPH) and cancer, respectively. Examination of the patient database showed that routine hospital histopathological diagnosis had been used to determine study and control groups. No PIN or proportion volumes of each type of disease state and each tissue volume were reported. Since the SCS method is dependent upon correct histopathological data the possibility that pathological en-
Figure 4. In vivo prostate MRI and MRSI at 1.5 T. A representative reception-profile corrected T2-weighted FSE axial image taken from a volume data set demonstrating a large tumor in the right midgland to base. The selected volume for hypointense lesion in right midgland (a) and overlay of spectral grid (b). c: Corresponding spectral array from b. Spectra in regions of cancer demonstrate dramatically elevated choline (d, red box) and a reduction or absence of citrate and polyamines relative to regions of healthy peripheral zone tissue (e, green box). The strength of the combined MRI/MRSI exam is demonstrated when changes in all three metabolic markers (choline, polyamines, and citrate) and imaging findings are concordant for cancer. (Reprinted from Kurhanewicz J. Swanson MG. Nelson SJ. Vigneron DB. Combined magnetic resonance imaging and spectroscopic imaging approach to molecular imaging of prostate cancer. Journal of Magnetic Resonance Imaging. 16:451–63, 2002, with permission from Wiley-Liss, Inc, a subsidiary of John Wiley & Sons, Inc.)

Figure 5. Comparison of prostate spectroscopy at 1.5 and 3 T. 1H-MR spectra from voxels in the peripheral zone of a prostate at 1.5 and 3 T are shown. The position of the voxel for which spectrum at 1.5 T (a) and at 3 T (b) originate is indicated (c). Spectra at 1.5 T (e) and at 3 T (f) correspond to the voxel (d) located in a slice in the base of the prostate 8 mm above the slice in (c). The citrate resonance is centered at 2.60 ppm. Notice the high spectral resolution at 3 T, e.g., in separating the lines of the citrate resonances and separation of choline and creatine peaks. (Reprinted from Füttner J, Scheenen T, Huisman H, Klomp D, van Dorsten F, Hulsbergen–van de Kaa C, Witjes A, Heerschap A, J. B. Initial experience of 3 Tesla endorectal coil magnetic resonance imaging and 1H-spectroscopic imaging of the prostate. Investigative Radiology; 39:671–680, 2004, with permission from Lippincott Williams and Wilkins.)
tities or variants may have been included in one classifier was considered. Furthermore, with the very complex zonal anatomy of the prostate the various mixtures of glandular and stromal tissues needed to be taken into account.

Using biopsy specimens, van der Graaf et al (39) identified decreased levels of spermine at 3.0 and 3.3 ppm as a marker of malignancy in the prostate, but found in vivo at 1.5 T that spermine was obscured in the 1D spectra by choline and creatine at 3.2 and 3.0 ppm, respectively.

The second study of prostate biopsies, also at 8.5 T (2), addressed the issue of specimen sampling for correlation with MRS data. The spectra were correlated with serially sectioned tissues, identifying an error rate of 8% in routine hospital procedures due to incomplete sampling of the tissue. No lipid suppression methods were used and typical ex vivo MRS spectra (8.5 T) from glandular BPH, stromal BPH, PIN, and invasive carcinoma are shown in Fig. 6. Also seen in Fig. 6 is a comparison of the spectra from excised tissues with different proportions of adenocarcinoma present (5% and 50%). There is a clear distinction on visual inspection between adenocarcinoma (50%), PIN, epithelial/glandular BPH, and glandular BPH. The presence of citrate in glandular BPH is consistent with the in vivo MRS of the prostate. However, no such citrate pattern is recorded for stromal BPH. Citrate can still be seen in the spectrum from PIN, as can the emergence of resonances consistent with choline and creatine. On visual inspection, the spectral separation of tissue with 5% adenocarcinoma from tissue containing only PIN was not easy. Resonances from lipid, amino acids, citrate, choline, and creatine are seen in the spectra from biopsy specimens containing small levels of adenocarcinoma and PIN, albeit at different levels. Once again, it can be seen that there is a gradation in the spectra from benign, stromal, and glandular BPH, through PIN, to frankly malignant tissue with increasing volumes of tumor.

The Swindle et al (2) study also divided the biopsy specimens from BPH into patients with and without cancer elsewhere in the prostate and compared these with adenocarcinoma specimens. When serial sectioned histopathology was used for correlation with MRS, a sensitivity of 100% and specificity of 94% was achieved. Depleted citrate and elevated choline measures alone were not accurate markers of malignancy since citrate levels remained high when only a small amount of malignant tissue was present (Fig. 6). A recently developed mathematical regression method (74) identifies small volumes of carcinoma (less than 50%) with accuracies approaching 100%. Thus the combination of different cell types (e.g., stromal, glandular, and zonal differences) can be documented and (combined with accurate histopathology) can offer a highly accurate diagnosis of malignancy in biopsy examinations at 8.5 T.

It would therefore appear that while histology remains the routine standard, spectroscopy could provide important biochemical information to improve our understanding of tumor development and progression in the prostate since, clearly, there are different signa-

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**Figure 6.** Spectroscopy on prostate biopsies. \(^1\)H MR (8.5 T) spectra of prostate biopsy specimens, 256 accumulations, sweep width 3597 Hz, and a pulse repetition time of 2.14 seconds. The water peak was suppressed by selective gated irradiation. **a:** Adenocarcinoma (50% of the tissue was made up of malignant tissue). **b:** Adenocarcinoma (5% of the tissue was made up of malignant tissue). **c:** Prostatic intraepithelial neoplasia (PIN). **d:** Stromal BPH (95% stromal, 5% glandular). **e:** Glandular BPH (85% glandular, 15% stromal) are compared. (Reprinted in part from Swindle P, McCredie S, Russell P, Himmelreich U, Khadra M, Lean C, L., Mountford CE. Pathologic characterization of human prostate tissue with proton magnetic resonance spectroscopy. Radiology 2003;228:144–151, with permission from the Radiological Society of North America.)
tures for glandular and stromal BPH, PIN, and adenocarcinoma.

There remain contentious issues with the in vivo experimental protocols for prostate. Inner voxel lipid suppression clearly impacts on the spectral profile of the prostate and comparison of non-lipid-suppressed spectra from the region of interest is warranted. The new mathematical regression methods developed by Somorjai appear to offer well-needed assistance in the interpretation of the chemical composition of potential multifocal and heterogeneous prostate disease. The chemical information allowing definitive and detailed pathology now needs to be collected and interpreted in a manner that will allow all pathology subtypes to be identified with high accuracy.

In vivo prostate spectroscopy is currently operational in many centers around the world, albeit with different protocols. With the significantly improved signal to noise ratio at 3 T it is now a matter of time before there are robust classifiers for automated pathological diagnosis and an accurate identification of the extent of the disease and the effect of the disease on the neurovascular bundles.

**CERVIX**

Cancer of the uterine cervix was chosen by the pathologist (P.R.) for the first $^1$H MRS study on clinical biopsies. The disease reflects the influence of mixed infections and high-risk human papillomavirus infections in these patients. The study reported on 51 women: nine were volunteers with benign uterine disease; 10 with CIN; and 32 with invasive malignant disease (28 with squamous cell carcinoma and four with adenocarcinoma). It would appear from this study that elevated lipid levels detected by MRS in vivo are independent of tumor load in the volume of tissue sampled. However, resonance peaks were all phased to choline and their chemical shifts were assigned relative to choline at 3.2 ppm. They reported that the sensitivity for detection of lipids in vivo using the single-voxel method was limited by technical and patient-related factors, including imperfect slice-selection profiles.

Potential developments in the preoperative diagnosis include use of ex vivo MRS on biopsy, material from proven cervical cancers to predict the presence of nodal spread, and use of in vivo spectroscopic imaging to predict the size and spread of the primary tumor.

**THYROID**

Thyroid nodules are very common, and the vast majority is biologically benign. The difficulty facing the clinician is selecting the few genuinely malignant thyroid nodules for surgery (76), when a correct diagnosis can only be made on excised material examined histologically. Fine-needle aspiration biopsy, although accurate for distinguishing papillary, medullary, and anaplastic carcinoma, is unable to distinguish benign from malignant follicular neoplasms. The criteria for malignancy are capsular or vascular invasion at the periphery of the neoplasm. This requires surgical removal of the entire tumor and extensive laboratory examination. Thus, the diagnosis of malignancy depends very much on sampling and is, at times, pure chance. This dilemma was editorialized by Ernest L. Mazzefarri (76).

**Intraoperative Biopsies**

In the first MRS study, tissue was obtained intraoperatively from 53 consecutive patients undergoing partial or total thyroidectomy for solitary thyroid nodules (28). Typical 1D $^1$H MR spectra (8.5 T) of follicular adenoma and follicular carcinoma are compared in Fig. 8. Visual inspection of the data and measuring the ratio of resonances at 1.7 and 0.9 ppm, MRS distinguished normal tissue from carcinomas (proven clinically or histologically) with an accuracy of 100% (28). When this resonance ratio was calculated for all follicular adenomas, several specimens grouped with the benign lesions and others with the carcinomas. One possible explanation is that adenomas with a malignant spectral pattern are in the process of malignant transformation. When the SCS method was applied to this thyroid data an accuracy of 100% was reported for normal thyroid vs. carcinoma (77).

It is important to recall that the specimens in this particular study were obtained intraoperatively, i.e., after ligation of blood supply to the thyroid. In order for this biopsy method to be of use it had to be extended to
FNAB obtained preoperatively. However, the thyroid is a very vascular organ and the FNAB specimens contained significant amounts of blood, which masked the diagnostic chemical signature (W. Mackinnon, C. Lean, P. Russell, L. Delbridge, P. Malycha, and C. Mountford, unpublished results).

**Thyroid Spectroscopy In Vivo**

There are now three reports of in vivo spectroscopy of the thyroid at 1.5 T (78) and 3 T (1). The group in Hong Kong (78) used a standard volume neck coil to show the feasibility of in vivo MRS at 1.5 T by examining eight carcinomas (including follicular, anaplastic, and papillary) and five apparently normal thyroid glands. A spectrum obtained from an anaplastic carcinoma (TE = 11005 msec) is shown in Fig. 9. The analysis centered on the presence or absence of choline to creatine ratios.

A special purpose-designed multiring surface coil was built for undertaking in vivo MRS of the thyroid at 3 T (79). A typical 3-T spectrum from a solitary thyroid nodule is shown in Fig. 10. The T1-weighted axial image of the thyroid lesion is compared with a spectrum of a thyroid biopsy of the same pathology but recorded at 8.5 T. The lesion is a benign adenoma as determined by MRS on the biopsy and confirmed histologically.

It can be seen that the in vivo spectra obtained at both 1.5 T and 3 T are directly comparable to those recorded at the higher field strength of 8.5 T and on biopsy material from patients with the same diagnosis. The development of the MRS method for determining accurate diagnosis would remove the necessity for thyroidectomy for many women. However, while this method shows potential, a large study involving serially sectioning the entire thyroid now needs to be undertaken and classifiers need to be developed.

**OVARY**

Ovarian cancers occur in one out of 57 women. The disease is commonly asymptomatic until it has metab-
tased with 70% to 75% of women presenting with advanced stage disease; the five-year survival for these women is only 20%. This poor overall outcome has created a bias towards aggressive therapy for all women with ovarian disease. Quality of life is important in deciding the extent of surgical procedures for patients. For ovarian cancer patients, this includes preserving fertility potential and minimizing the negative fertility effects of cancer treatment in young women (80).

In particular, conservative surgical management of early-stage epithelial ovarian cancer (81) offers much to the patient when the disease is staged and typed accurately. It would be important for those patients with genuinely benign disease of those with so-called “low malignant potential” (LMP) tumors, which may or may not be malignant, if their diagnosis could be established prior to surgery, thus allowing more confident triaging for appropriate therapy.

The LMP tumors, which frequently occur in young women, account for 14% of ovarian surface epithelial/stromal neoplasms. These commonly encountered “surface epithelial stromal tumors” (82) have notionally benign and malignant counterparts that are separated by a quite arbitrary histological criteria, which may not correlate with the clinical outcome for the patient.

Thus no guidelines currently exist, at the interface between benign and malignant, which provide for a reliable prediction of the biological potential in a given case of ovarian epithelial neoplasia. Many women undergo unnecessary surgery and therapy for tumors that have no malignant potential (82). The extra ovarian “implants” associated with such LMP neoplasms are often thought of as metastases, leading to unnecessary treatment in many cases. Recent molecular genetic studies (83,84) have cast doubt on the premalignant potential of the proliferating (LMP) serous tumors, making it more critical that they be accurately distinguished from their frankly malignant counterparts.

**Intraoperative Biopsies**

In an initial study of approximately 70 cases, it was shown that MR spectral differences distinguished between subsets of ovarian epithelial/stromal tumors (23). Typical 1D MR spectra from histologically normal, benign, proliferating (LMP) and malignant ovarian tumors of serous and mucinous types were compared in this report. In the malignant category, visual inspection showed that while the malignant serous and mucinous tumors had very similar 1D MR profiles, the benign category showed slight differences. In the LMP category, mucinous tumors had a far more active chemical profile than serous LMP tumors, with the mucin being clearly visible at around 3.8 ppm. An explanation is that some mucinous LMP tumors are already exhibiting MR evi-

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**Figure 8.** $^1$H MR (8.5 T; 37°C) spectra from follicular carcinoma (a); benign follicular adenoma (b). Spectra were acquired with 256 accumulations, residual water was suppressed using gated irradiation. Diagnostic resonances at 0.9 ppm (CH$_3$, lipid) and 1.7 ppm (CH$_2$, lysine) are denoted. +N(CH$_3$)$_3$ = N-trimethyl from choline containing metabolites, HOD = residual water. (Reprinted from Mountford CE, Doran ST, Lean CL, Russell P. Cancer pathology in the year 2000. Biophysical Chemistry, 1997; 68:127–135, with permission from Elsevier.)

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**Figure 9.** Anaplastic carcinoma of the thyroid in vivo. a: Proton MR spectrum acquired at TE = 136 msec from the primary tumor in a patient with an anaplastic carcinoma of the right lobe of the thyroid. The nominal voxel volume was 36 cm$^3$. Prominent peaks detected were Cho (3.2 ppm), Cr (3.03 ppm), fatty acid (FA) (–CH$_2$) (1.30 ppm) and FA (–CH$_3$) (0.90 ppm). b: Transverse T1-weighted MR image shows the position of the volume of interest for spectroscopy represented by the white square box placed within the primary tumor. (Reprinted from King AD, Yeung DKW, Ahuja AT, Tse GMK, Chan ABW, Lam SSL, van Hasselt AC. In vivo 1H MR spectroscopy of thyroid carcinoma. European Journal of Radiology, 2005; 54:112–117, with permission from Elsevier.)
Adenoma–Carcinoma Sequence as Monitored by 2D Correlated Spectroscopy

The 2D correlated spectroscopy (COSY) method was developed by Ernst (43) and first applied to cells in 1984 (14). Typical 2D COSY spectra of normal ovarian tissue and serous carcinoma are shown in Fig. 11. The COSY spectrum of the poorly differentiated serous carcinoma (Fig. 11b) is typical of carcinoma from many organs (14,21,27,86). An increase in chemical activity can be seen in Fig. 11 from normal ovary to carcinoma of the ovary, which is also consistent with the spectra recorded for other organs exhibiting an adenoma–cancer sequence.

One particular part of the COSY spectrum that has provided a valuable insight into the presence of the adenoma–cancer sequence is indicated on Fig. 11b. This spectral region, F1 = 3.9–4.5 ppm and F2 = 1.0–1.6 ppm, contains the methyl-methylene coupling from lactate (1.33–4.12 ppm) and cross-peaks from cell-surface fucose (21).

It is this region of the spectrum that has identified alterations associated with loss of cellular differentiation and an increase in cell surface fucosylation (22,27) a phenomenon well documented by immunohistochemical methods (87–90). The increasing complexity of the COSY fucosylation spectral pattern with tumor development and progression has been shown previously for colon (22,27) and breast (1). The increasing complexity of the COSY fucosylation spectral pattern with ovarian tumor development and progression is shown in Fig. 12, where the expanded methyl-methylene coupling region from the COSY spectra are shown for normal ovary, benign tumor, proliferating tumor, well-differentiated carcinoma, moderately differentiated carcinoma, and poorly differentiated carcinoma. Analysis of the 2D COSY data showed that of Fucl, IIb, or III were present in spectra from carcinoma that were poorly differentiated.

Of the LMP ovarian tumors examined, three of nine (one serous and two mucinous) generated cross-peak Fucll in addition to FucI, FucII, and FucIIb and are therefore spectroscopically consistent with being malignant. In stark contrast the remaining six LMP tumors generated a single cross-peak at Thr/FucI spectral frequency as seen in Fig. 12a. Fibromas, benign epithelial tumors, and normal tissue also generated a single cross-peak at the Thr/FucI spectral frequency as seen in Fig. 12a. Thus three of the LMP tumors were chemically akin to the malignant tumors, and six more closely resembled benign tumors.

In Vivo at 3 T

In vivo spectroscopy and imaging of the ovary at 3 T can provide diagnostic information preoperatively on patients with ovarian masses. The 3-T pelvic image of a patient with a suspected ovarian cancer is shown in Fig. 13 (91). There is a mixed cystic and solid lesion measuring 4 cm in diameter. Two-dimensional CSI was undertaken in the region identified by the box. The spectral information obtained from the voxel placed over the solid lesion is shown to the left. This spectrum contains choline (3.2 ppm), creatine (3.0 ppm), other metabolites and at 0.9 (not shown on figures), 1.2 ppm assigned to the methyl and methylene of lipid and is consistent with a malignant tumor. The voxel on the right is placed on the cystic component and the resultant spectrum is to the right. This spectrum shows a small contribution from choline, indicating the presence of malignant cells. The ex vivo (8.5 T) (not shown) and in vivo (3 T) spectra are very similar. In the 2D spectra (Fig. 11), the region 3.8–4.5 and 1.0–1.6 ppm shows cell surface fucosylation patterns consistent...
with level of dedifferentiation (1,23). The independent histopathological examination confirmed the spectral diagnoses.

Ovarian tumors tend to present clinically when quite large (10 cm in diameter or larger). This makes the implementation of single voxel relatively easy and thus amenable to routine clinical examination in vivo at 3 T. The accuracy of the spectroscopy method in vivo remains to be determined.

ESOPHAGUS

Endoscopic Biopsy

Adenocarcinoma of the lower esophagus in the Western world (92) is rising in incidence and accounts for 40% of esophageal carcinomas in males (92,93).

Esophageal carcinoma is thought to be caused from gastric reflux and a condition known as Barrett’s esophagus is considered to be a precursor (94), with a 40- to 50-fold increased risk of developing malignancy. Histopathology distinguishes normal from invasive carcinoma of the esophagus with a high level of accuracy. Histopathology is not, however, able to provide an accurate prediction of the potential of dysplastic Barrett’s epithelium (95,96).

In a study investigating 72 consecutive patients, 29 non-cancer-bearing and 43 cancer-bearing, MRS analyzed by SCS distinguished normal epithelium from esophageal carcinoma and from Barrett’s esophagus. Typical 1D $^1$H MR spectra at 8.5 T are shown in Fig. 14. $^1$H MRS combined with the SCS method distinguished normal Barrett’s epithelium from carcinoma with an accuracy of 100%, normal from Barrett’s with an accuracy of 100%, and Barrett’s vs. carcinoma at 96% (19). The spectra from Barrett’s and adenocarcinoma contained a relatively intense choline resonance.

Of importance are the spectra from Barrett’s epithelium from a non-cancer-bearing patient and from a cancer-bearing patient with the corresponding histopathology in Fig. 14. Histologically, these tissues are indistinguishable, yet the MR spectra group the specimens from cancer bearing patients with adenocarcinoma. There is evidence supporting the existence of an adenoma–carcinoma sequence in the esophagus (97,98). These MRS data support the MRS method being able to identify field change consistent with the presence of an adenoma–carcinoma sequence as reported for the thyroid and colon. The MRS study on esophageal biopsies has been in progress for over seven years. Patients are informed of the research outcome of the MRS analysis and the number of those patients identified as having tissue committed to malignancy are now presenting for surgery (Falk G, Doran S, Phillips J, Lean C, Russell P, Mountford C, unpublished results). The test is ready for clinical acceptance testing to ascertain its ability to predict the biological potential of Barrett’s esophagus on biopsy.

To date there has been no recorded attempt to develop this technology for in vivo MRS diagnosis.

TRANSLATION OF SPECTROSCOPY INTO THE CLINIC

For spectroscopy to be effectively transferred from an academic setting into routine hospital use there needs to be a strong working collaboration between the multidisciplinary team and a commercial entity. Education
of all participants is an important means of ensuring effective translation into the clinic.

BIOPSY PROGRAM

Housekeeping issues, such as the plastic used for collection vials and syringes, and storage conditions for specimens, are of paramount importance (99). As described earlier, biopsy techniques, particularly for the FNAB, are important as at least $5 \times 10^6$ cells are needed for adequate SNRs to be obtained during sample testing. However, once these technical elements are under control, the data will, in the future, be able to be analyzed automatically by robust classifiers.

IN VIVO MRS

The technical difficulties have been well described for neurospectroscopy by Roland Kreis (100), and these difficulties apply equally well to other organs. The difficulties initially experienced by the UCSF team in extending their prostate program to other centers has now been overcome due to a closer working relationship with a commercial partner and the significant time and effort expended by Dr. Kurhanewicz and his colleagues in assisting other sites. The same care and time will now need to be given for breast spectroscopy to come of age in vivo.

Technical variables such as the coils for each organ (whether they are transmit-receive or receive-only), are of paramount importance for body spectroscopy. Currently, there is a plethora of coils available for the breast and it remains to be determined which coils are suitable for spectroscopic analysis in addition to MRI. Traditionally, coil manufacturers have set their sights on high quality images and ignored spectroscopy. This is now changing.

CONCLUSIONS

Proton MRS is now ready to be introduced into the pathology laboratory, initially for the breast, where it can not only provide a diagnosis but also prognostic indicators. The technology is also ready to commence acceptance testing to ascertain its ability to predict the biological potential of Barrett’s esophagus lesions.

The combination of MRI and MRS in vivo with correlative MRS on biopsy specimens offers great accuracy for the diagnosis and prognosis of some human cancers. Currently, the only body organs for which this can be undertaken routinely are the breast and the prostate.

In the case of prostate, multifocal disease is common and the MRS method offers a method for mapping multifocal disease. For breast, spectroscopy in vivo can identify regions of abnormal pathology where ultrasound and computed tomography (CT) can fail. For both breast and prostate the capacity to undertake image guided biopsy using 3D CSI will significantly improve the preoperative diagnostic accuracy. It remains
to be clarified whether spectroscopy is more accurate at determining the extent of disease in these organs compared with MRI, as has been shown for the brain (101).

The proof that MRS can monitor disease as well as predict tumor progression will be in the next five years, a mere 30 years after the initial observation. Time will provide testament to the accuracy of MRS both on biopsy and in vivo in the cancer clinic. If correct, the spectroscopy technology will also apply to other areas such as cardiac disease, and diagnosis of causation of pain.

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