Applications of magnetic resonance spectroscopy in radiotherapy treatment planning

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ABSTRACT. Following advances in conformal radiotherapy, a key problem now facing radiation oncologists is target definition. While MRI and CT provide images of excellent spatial resolution, they do not always provide sufficient contrast to identify tumour extent or to identify regions of high cellular activity that might be targeted with boost doses. Magnetic resonance spectroscopy (MRS) is an alternative approach that holds great promise for aiding target definition for radiotherapy treatment planning, and for evaluation of response and recurrence. MRS is able to detect signals from low molecular weight metabolites such as choline and creatine that are present at concentrations of a few mM in tissue. Spectra may be acquired from single voxels, or from a 2D or 3D array of voxels using spectroscopic imaging. The current state of the art achieves a spatial resolution of 6–10 mm in a scan time of about 10–15 min. Co-registered MR images are acquired in the same examination. The method is currently under evaluation, in particular in brain (where MRS has been shown to differentiate between many tumour types and grades) and in prostate (where cancer may be distinguished from normal tissue and benign prostatic hypertrophy). The contrast achieved with MRS, based on tissue biochemistry, therefore provides a promising alternative for identifying tumour extent and regions of high metabolic activity. It is anticipated that MRS will become an essential tool for treatment planning where other modalities lack the necessary contrast.

Magnetic resonance spectroscopy (MRS) is a non-invasive technique for measuring biochemicals in tissue. It uses the same general principles and equipment as its widely used partner, MRI. However, while MRI builds images using signals from 1H nuclei in tissue water (and sometimes lipid), present at concentrations of approximately 35 M, MRS is used to measure signals from magnetic nuclei (usually 1H, but 31P has also been extensively studied) of tissue metabolites such as choline, creatine, and lactate that are present at much lower concentrations (typically of the order of a few mM). Example spectra from normal brain and from brain tumour are shown in Figure 1, to illustrate the type of data that are obtained. Further details of specific metabolites detected in brain and prostate are listed in Table 1 and discussed in the corresponding sections towards the end of the article. More detailed introductions to the use of in vivo MRS may be found in the literature [1, 2].

The frequency of MRS signals depends only on the gyromagnetic ratio (γ) of the nucleus (which is a constant for a particular nuclear species, e.g. γ(1H)=2.675 × 10⁸ rad/T/s; γ(31P)=1.083 × 10⁹ rad/T/s), and on the local magnetic field, Blocal, experienced by the nucleus (Equation (1)).

\[ f(\text{Hz}) = \gamma(\text{rad/T/s}) B_{\text{local}}(\text{T}) / 2\pi \]  

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the bonding to neighbouring nuclei. Thus, different nuclei of the same species within a single molecule produce distinct peaks within a spectrum (unless they are "magnetically equivalent"), which usually permits different compounds of biochemical interest to be distinguished. In addition, one can study compounds naturally present in tissue without the requirement for specific labelling. The major disadvantage of MRS is that the signals from metabolites are relatively small, so that compared with MRI much larger "pixels" or "voxels" are required to obtain an adequate signal-to-noise ratio. Methods for data acquisition and some related issues are described in the section on "Technical Issues" below.

In contrast to CT and MRI, which provide morphological information about tissue, MRS gives biochemical information (Figure 1). Since the biochemistry of tumours is substantially different from that of normal tissue, MRS has the potential to aid identification of tumours when there is insufficient contrast in the morphological image. In particular, it can be used for differential diagnosis, both between tumours and benign pathology (e.g. prostate cancer and benign prostatic hypertrophy [3]), and between different tumour types (e.g. different brain tumours [4]). Since MR spectra are acquired using MRI scanners, they are automatically co-registered with MR images, which may then themselves

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Table 1. Brief details of some metabolites seen in $^1$H spectra of tissues

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Chemical shift of main peak (ppm)</th>
<th>Number of equivalent $^1$H nuclei</th>
<th>Multiplicity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cho</td>
<td>3.2</td>
<td>9</td>
<td>singlet</td>
<td>&quot;Cho&quot; includes contributions from choline, phosphocholine, glycerophosphocholine and other trimethylamines. These metabolites are involved in cell membrane lipid synthesis and breakdown, and are also affected by signalling pathways that can be upregulated in tumours. Since 9 magnetically-equivalent protons contribute to this peak, relatively low concentrations produce a measurable signal</td>
</tr>
<tr>
<td>Cr</td>
<td>3.02</td>
<td>3</td>
<td>singlets</td>
<td>&quot;Cr&quot; includes creatine and phosphocreatine, which are both involved in energy metabolism</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.33</td>
<td>2</td>
<td>doublet</td>
<td>Lactate is a product of anaerobic glycolysis, a further aspect of energy metabolism, often being found in necrotic areas</td>
</tr>
<tr>
<td>Lipids</td>
<td>1.3, 0.9 etc.</td>
<td>2</td>
<td>singlet</td>
<td>Often found in necrotic regions</td>
</tr>
<tr>
<td>myo-Inositol (mI)</td>
<td>3.52</td>
<td>2</td>
<td>doublet of doublets</td>
<td>This can be detected in brain using shorter echo time acquisitions. Understood to be an essential ingredient for cell growth, an osmolite, and a storage form of glucose</td>
</tr>
<tr>
<td>NAA</td>
<td>2.01</td>
<td>3</td>
<td>singlet</td>
<td>NAA is considered to be a neuronal marker, so only present in brain</td>
</tr>
<tr>
<td>Citrate</td>
<td>2.6</td>
<td>1</td>
<td>multiplet</td>
<td>This is synthesized and accumulated by normal prostate epithelial tissue</td>
</tr>
</tbody>
</table>
be co-registered with planning CT scans in the normal way if required (see also the paper by Kessler in this issue).

In the context of radiotherapy, MRS has several potential applications. These include:

1. Identifying tumour extent and metabolically active regions to aid targeting of radiotherapy. This includes distinguishing tumour from normal tissue, benign lesions, or radiation necrosis.
2. Evaluating response to treatment
3. Identifying recurrence

With the advent of new conformal techniques for radiotherapy, target definition has become one of the main issues in further improving the effectiveness of radiotherapy treatment. In particular, it is necessary to delineate accurately the extent of tumour tissue, and one may also wish to identify regions of high clonogenic cell density, which could be targeted with boost doses of radiotherapy using either intensity-modulated external beam radiotherapy or brachytherapy. Conventional imaging methods are often limited in this respect. For example, in the prostate, MRI has better soft tissue contrast than CT [5], but still only has a positive predictive value of 50% [6]. In addition, owing to the different sources of contrast in the two cases, the boundaries shown are often different [7, 8]. Since MRS measures tissue biochemistry, it has the potential to identify abnormalities and the nature of the abnormality more selectively than morphological imaging can achieve.

Current status of MRS in target planning for radiotherapy

$^1$H MRS packages are currently available for most commercial clinical MR scanners, usually including both single-voxel localization techniques and MR spectroscopic imaging (MRSI; for more details see subsection on localization strategies below). However, owing to the extra expense, and the time required to perform MRS examinations, many hospitals choose not to have this option. The packages are increasingly easy to use, but previous experience is important to achieve good results, and to understand the reasons for technical failures. Assuming diagnostic MR images have already been acquired, the extra time required for $^1$H MRS is typically a few minutes for single-voxel techniques, and 10–15 min for MRSI. Some software for spectral processing is usually provided, but for quantitative work many users choose to export their data to specialist spectral processing software. MRS of nuclei other than $^1$H is less used owing to the requirement for additional hardware and (usually) longer scan times.

While MRS has great potential to aid target identification in radiotherapy treatment planning, it is not currently used to any significant extent for this purpose. This is partly because it is only recently becoming available in a form that is relatively easy to use. In order to alter current treatment protocols to utilize MRS, the following steps need to be achieved:

- to validate spectroscopic observations against histology
- to demonstrate a correlation between MRS observations and survival or local recurrence based on existing treatments, and hence to define a measure of abnormality in different cases to use for delineating the treatment target volume
- to demonstrate improvement in survival in well-defined clinical trials
- to make the current state-of-the-art measurements routinely and robustly available, with tools to aid interpretation of spectroscopic data
- to educate the radiotherapy community

To improve spatial resolution (currently about 6–10 mm) would be helpful but probably not essential, as practical radiotherapy techniques will include a margin of this order. As usual, there is a trade-off between signal amplitude and spatial resolution, so improving spatial resolution requires an improved methodology to increase the sensitivity of signal detection.

Some of these issues are already being addressed, in particular in studies of the brain and prostate (see below).

Technical issues

Magnetic field strength

Most clinical MR studies are performed at a magnetic field strength of 1.5 Tesla and most published MRS work, including most of that referred to in this review, has been performed at this field strength. In research environments, 3 T scanners are becoming increasingly used, as the signal-to-noise ratio and spectral resolution both increase (approximately linearly) with field strength. The doubling of signal-to-noise ratio at 3 T can be traded for a 50% reduction in voxel volume (i.e. a reduction in voxel size of (0.5)$^{0.8}$ = 0.8 in each spatial dimension) compared with 1.5 T scanners, but otherwise the functional information is very similar. Systems with much higher magnetic fields for human studies at 7 T and 8 T have been installed in a handful of research sites. However, these are very expensive, present a large number of technical challenges to operate (in particular the correspondingly higher gradient amplitudes and radiofrequency power deposition can exceed safety limits unless duty cycles are reduced) and generally (except in the brain) do not produce good diagnostic images owing to dielectric resonance effects [9]. Problems with image distortion (see below) will also increase at the higher fields.

Shimming

The sharpness of spectral lines depends on the homogeneity of the magnetic field. This is because a spread in values of the local magnetic field leads to a spread of frequencies (Equation (1)). The effect of placing a sample into a homogeneous field usually disturbs the field homogeneity. MR systems are equipped with a series of “shim coils” which create small additional magnetic fields that can be adjusted to help counteract this effect. This “shimming” process is usually performed at the beginning...
of each MRS study, and takes a few minutes. On current MR systems the process is normally automated.

**Magnetic nuclei studied**

Because clinical MR scanners are designed to detect signal from $^1$H nuclei, only additional software is required to acquire $^1$H MR spectra. In addition $^1$H is the most sensitive magnetic nucleus, and hydrogen is present in nearly all biologically relevant compounds. A list of metabolites seen in $^1$H spectra of several tissues is given in Table 1. More details for brain metabolites can be found in the work of Govindaraju, Young and Maudsley [10]. If nuclei within a molecule are “equivalent”, then they all contribute to the same peak. Peaks appear as multiplets (doublets, triplets etc.) when the nucleus of interest is covalently bound directly or through other nuclei to another (but non-equivalent) magnetic nucleus (e.g. $^1$H, $^{31}$P etc.). This splitting (known as J-coupling) decreases as the number of bonds between the nuclei increases.

In order to study compounds at millimolar concentrations, it is necessary to suppress the large signals from tissue water, and also sometimes from lipids. Suppression techniques are available to accomplish this.

For comparison of spectra it is necessary to have a reference frequency. $^1$H MRS studies in solution often include tetramethylsilane (TMS) for this purpose, with a frequency (or “chemical shift”) set to 0 ppm. TMS is used because it is stable and produces a sharp single peak. For consistency, the positions of spectral peaks in vivo are still expressed relative to TMS, even though it is not present in tissue; other peaks that are present, and which have shifts that are relatively independent of environmental conditions such as pH and metal ion concentration etc., are used as an internal secondary reference.

Other nuclei that may produce useful MR spectra are $^{31}$P, $^{13}$C and $^{19}$F. Phosphorylation is an important biochemical process, and several compounds of key importance therefore contain phosphorus. Several metabolites seen in $^{31}$P MR spectra are affected by signalling pathways up-regulated in cancer [11, 12] and therefore $^{31}$P MRS could play an important role in target identification and evaluation of therapy, especially of new therapeutic agents. One study [13] in soft tissue sarcoma treated with thermoradiotherapy has shown strong correlations between pre-treatment MRS data (in particular the ratios of phosphodiesters and of phosphomonoesters to inorganic phosphate) and pathological complete response. However, since $^{31}$P has only 6% of the sensitivity of $^1$H at a given field strength, larger voxels are required to obtain an adequate signal-to-noise ratio. In addition, extra hardware (RF amplifiers, filters, RF coils) is required that is tuned to the $^{31}$P resonance frequency.

Currently, little work has been performed using $^{13}$C nuclei (owing to only 1% natural abundance and a lower sensitivity). While no endogenous metabolites contain MR-visible fluorine, several studies have demonstrated the value of $^{19}$F MRS in following drug metabolism [14].

**Localization strategies**

MRS localization techniques fall into two groups. Single-voxel methods include those known as PRESS [15], STEAM [16] and ISIS [17], in which data are acquired from a single voxel positioned using MR images. The alternative is MRSI, also known as chemical shift imaging, (CSI) [18], in which a matrix of spectra are acquired either over a plane (2D-MRSI) or a volume (3D-MRSI). The choice of localization method affects the efficiency of signal detection from within the target region and the contribution of unwanted signals from outside the volume (contamination). The effectiveness of the shimming method in optimizing the magnetic field homogeneity in the selected region, the effectiveness of suppressing the very large signals from water (and sometimes from lipid present in the selected volume) and the ability to suppress signals from outside the volume of interest can also affect spectral quality. In the brain, cerebral spinal fluid flowing into the selected region can also adversely affect shimming and water suppression. Sequence timing parameters determine the $T_1$ and $T_2$ weighting of the sequence, and also affect modulation of coupled spins. Common echo times (TE) are 270 ms (minimizing contributions from lipids and macromolecules), 135 ms (inverting the lactate doublet) and 20 ms (maximizing signal from macromolecules and lipids). A summary of the merits of single voxel MRS relative to MRSI is given in Table 2. For most applications in radiotherapy treatment planning use of MRSI will be preferred, with single voxel methods restricted to cases where it is required to characterize a single well-defined lesion, when time is short, or when adjacent structures make it hard to shim over larger regions.

**Registration and motion issues**

The volumes selected for acquisition of the MR spectra are positioned using MR images acquired in the same examination. The MRS and MRI data are therefore inherently co-registered. However, although MR images have better soft tissue contrast compared with CT images, they have two major disadvantages for radiotherapy treatment planning – they suffer distortions and they do not intrinsically provide electron density information. These issues have been extensively discussed elsewhere [19]. There are two approaches to solve this problem. One approach under development is to correct the distortions [20, 21] and to make use of the MR images to estimate the electron density information [22]. The alternative is to co-register the MR images with CT images – usually using either external fiducial markers that can be seen by both imaging modalities, or using methods such as “mutual information” [23] based on the features within the images (see also the paper by Kessler in this issue). It is also necessary to ensure that the MR scanner is equipped with a flat-topped couch (similar to that used for treatment), and with suitable laser positioning beams.

Motion during the scan is a potential problem. Being rigid, immobilization of the head is fairly straightforward, although there is still some residual pulsatile motion from the circulation of cerebral spinal fluid. Other parts of the body are more challenging. For studies of the prostate it is common to use agents like Buscopan to reduce peristalsis. Data acquisition can be synchronised to the heart or breathing cycle using cardiac or respiratory triggering, while “navigator echoes”, an MR
technique that identifies a column of tissue and monitors movement along this column [24], may also be used. It is not possible to eliminate motion entirely and, as with conventional CT techniques, this must be remembered when prescribing the margins for irradiation. As the application of MRI and MRS for radiotherapy planning develops, it is likely that use of immobilization devices will be evaluated in more detail.

Validation

Before radiation oncologists have the confidence to alter treatment plans based on MRS data, they need evidence that the abnormality detected using MRS represents tumour. The “gold standard” for comparison is histopathology (where available). In some cases, such as prostate (see below), studies have been performed where the prostate has been removed very shortly following the \textit{in vivo} MRS examination, so that MR spectra can be directly compared with histopathology of the whole gland. This is the best possible situation for validation. In other cases biopsy samples are available, although it is often difficult to be certain of their precise location. Results of some of these studies are described below. Interpretation of the \textit{in vivo} spectra can be aided by high-resolution \textit{ex vivo} MRS of intact tissue samples acquired using magic angle spinning (MAS) at high magnetic fields [25]. Such spectra have much higher spectral resolution and sensitivity than spectra acquired \textit{in vivo}, and permit detection and quantification of metabolites that in the \textit{in vivo} spectrum are either at too low a concentration or are in regions of spectral overlap. Other imaging methodologies (MRI, PET etc.) give supporting evidence, but may themselves lack the necessary validation.

\textbf{Example 1: MRS and brain cancer}

\textbf{General issues regarding diagnosis and treatment}

There is a strong incentive for non-invasive assessment of brain cancer, particularly in children, and especially where sequential evaluations are required. In managing patients, the first priority is diagnosis, currently usually confirmed by histology, although this suffers from known limitations of sampling error and tumour heterogeneity. Guidance of biopsy is therefore also important. Assessment of tumour grade is important in defining treatment – this is usually limited to one time point, either at biopsy or during tumour debulking. Assessment of response following treatment is of increasing value, so that the treatment can be modified according to the response observed.

MRI appearance and location alone have limited power to differentiate brain tumours. In particular, early stage disease and infiltrative disease may have little impact on the blood–brain barrier, limiting the sensitivity of contrast-enhanced MRI and leading to the requirement for an alternative method to identify tumour boundaries. Identification of residual disease from necrosis can also be difficult with MRI. The metabolic profile provided by MRS offers considerable potential to distinguish different tumour types and grade.

Most MRS measurements of tumours have employed the $^1$H nucleus, owing to its greater sensitivity. $^1$H MRS

\begin{table}[h]
\centering
\caption{Comparison of single voxel techniques with spectroscopic imaging}
\begin{tabular}{|l|l|l|}
\hline
 & Single voxel & Spectroscopic imaging \\
\hline
1. Specification of VOI & & \\
\hspace{1em} No. of volumes & 1 & Typically $8 \times 8 \times 8$ (3d) or $16 \times 16$ (2d) \\
\hspace{1em} Voxel specification & Must be specified before measurement & Many voxels available \\
\hspace{1em} Voxel shape & Dimensions adjustable; also tilt & Grid shift can be performed retrospectively \\
2. Voxel integrity & & Orthogonal (usually square grid) \\
\hspace{1em} Edge definition & That of slice profile & Point spread function effects \\
\hspace{1em} Chemical shift & Yes – in each direction & Not present \\
\hspace{1em} displacement artefact & & \\
3. Sensitivity & $T_2$ (STEAM, PRESS) & $T_2$ (in STEAM and PRESS pre-localized implementations) \\
\hspace{1em} Relaxation losses & & Phase-encoding loses approximately 13% in each spatial dimension \\
\hspace{1em} Other losses & Imperfect RF pulse profiles and flip angles & Sometimes poor. Addition of small voxels does not recover SNR of the larger corresponding voxel \\
\hspace{1em} Conformation to target & Usually good & \\
4. Other aspects & & \\
\hspace{1em} Minimum number of acquisitions & 1 & Many (e.g. 512 for $8 \times 8 \times 8$ csi) \\
\hspace{1em} RF coils – uniform transmit & Good & Good \\
\hspace{1em} coils and surface coil receiver & & \\
\hspace{1em} RF coils – transmit/receive & ISIS works well provided adiabatic RF pulses & The basic implementation works well if an adiabatic excitation pulse is used; additional localization (slice or volume selection) have same problems as PRESS and STEAM \\
\hspace{1em} surface coil & & \\
\hline
\end{tabular}
\end{table}

VOI, volume of interest; RF, radiofrequency; SNR, signal to noise ratio.
of brain tumours has developed rapidly due to the less exacting technical requirements of spectroscopy in the brain compared with measurements elsewhere in the body. In particular, it is easier to control motion and most parts of the brain are fairly homogeneous, making it easier to achieve a good shim. Care should be taken with MRS when using contrast agents as this can broaden the choline peak, without affecting peak area. However, if shimming takes place immediately prior to MRS acquisition, this effect can be minimized or eliminated [26].

Features of $^1$H brain tumour spectra

Spectra from brain tissues (Figure 2) include contributions from total creatine (Cr) (3.94 ppm and 3.01 ppm), total choline (Cho) (3.22 ppm), N-acetyl aspartate (NAA) (2.01 ppm), myo-inositol (mI) and glycine (3.55 ppm), lactate (Lac) (1.35 ppm), alanine (Ala) (1.47 ppm), contributions from a range of lipid resonances (lip) (0.9 ppm, 1.3 ppm), broad resonances due to macromolecules (2.05–2.8 ppm, 5.4 ppm) and poorly resolved amino acids such as glutamate and glutamine (Glx) (approximately 2.3 ppm). In tumours other resonances may be present, or may contribute to peaks, but their contribution can be hard to confirm unless tissue specimens are available.

Application in differential diagnosis and assessing the grade of brain tumours

While MRI is an essential part of the evaluation of cranial neoplasms, indicating location, morphology, boundary definition and physiological characteristics, there remains considerable overlap between the appearance of primary tumours, metastases and radiation necrosis [27–33]. However, as illustrated in Figure 2, MR spectra do display differences between tumour types. For example, compared with normal brain, MR spectra of astrocytomas show elevated Cho, reduced Cre and significantly reduced NAA. A summary of the characteristics found in some common brain tumours is given in Table 3. More details can be found in Howe et al and Devos et al [28, 34]. While visual inspection of spectra can be helpful, there is considerable interest in automated evaluation of multiple spectral lines [29, 34, 35]. In particular this permits comparison of new spectra with those from tumours of known histology, provided the spectra are acquired under similar conditions (magnetic field, sequence timing parameters etc.). This approach is showing promising indications of improved discrimination of tumour type and grade. Since there are many tumour types, some of which are relatively rare, effective databases require many samples, thus necessitating multi-centre cooperation.

**Figure 2.** Mean and standard deviation (vertical lines) of normalized STEAM (echo time (TE)=30 ms) spectra acquired from a number of subjects: Normal white matter ($n=6$); meningioma ($n=8$); astrocytoma grade II ($n=5$); anaplastic astrocytoma ($n=7$); glioblastoma ($n=13$). Published with permission [28].
Therapeutic guidance, assessment of response and recent developments

Recently there has been increasing interest in using functional imaging techniques, together with metabolic imaging using MRS [36] to aid target definition in radiotherapy, for example to identify areas of infiltration not evident with MRI. Figure 3 illustrates an example where the boundaries of the lesion detected using MRS (based on levels of Cho and NAA) are quite different from those suggested by contrast-enhanced MRI [37]. In a further study [38] of patients with high grade glioma who were scanned following surgery (but prior to radiotherapy), MRS indicated areas of residual abnormality that did not enhance with contrast at that time, but of which a proportion did enhance subsequently at follow-up. In this study, MRS volumes were also greater than the $T_2$ weighted area of abnormality. A study of survival following gamma knife surgery [39] showed that patients with poor overlap (<50%) between treatment volume and volume of metabolic abnormality had reduced survival compared with those having good overlap.

Elevated Lip/Cre and Lac/Cre in the peri-tumoural region of high grade glioma may help to identify patients at risk of recurrence [40]. It is suggested that areas of relatively high Cho/NAA may indicate high cellular activity, and hence radio-sensitivity, and Lac may indicate hypoxic areas with reduced radio-sensitivity [37, 41]. The technique can also be helpful in identifying areas missed by radiation fields, and in separating

<table>
<thead>
<tr>
<th>Tumour type</th>
<th>$^1$H MRS characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>All tumour types</td>
<td>Low NAA, and reduced Cr</td>
</tr>
<tr>
<td>Astrocytomas</td>
<td>Elevated Cho, reduced Cre and significantly reduced NAA. With increasing WHO grade,</td>
</tr>
<tr>
<td></td>
<td>methylene lipid (1.3) and lactate may be seen, indicating necrosis. Methylene levels</td>
</tr>
<tr>
<td></td>
<td>correlate with contrast enhancement in MR images, and may allow the transformation from</td>
</tr>
<tr>
<td></td>
<td>low to high grade to be detected prior to focal contrast enhancement [63]. Cho may also</td>
</tr>
<tr>
<td></td>
<td>increase with the increased proliferative activity of higher grade tumours. Glioblastoma</td>
</tr>
<tr>
<td></td>
<td>multiforme has the same spectral pattern as metastasis</td>
</tr>
<tr>
<td>Low grade tumours</td>
<td>May show increased ml</td>
</tr>
<tr>
<td>Oligodendroglia and mixed</td>
<td>ml may also be elevated, together with Cho</td>
</tr>
<tr>
<td>oligoastrocytoma</td>
<td>Low [Cr] and [mIG], increased Cho, although there may be low levels of lipids and a</td>
</tr>
<tr>
<td></td>
<td>characteristic presence of alanine (1.47)</td>
</tr>
<tr>
<td>Metastasis</td>
<td>Similar features to astrocytomas, with increased lipid if necrosis is present. However,</td>
</tr>
<tr>
<td></td>
<td>they may have a distinct spectroscopic boundary. Thus the presence of elevated choline/</td>
</tr>
<tr>
<td></td>
<td>creatine ratio in the peritumoural region may suggest high grade glioma rather than a</td>
</tr>
<tr>
<td></td>
<td>solitary metastasis [64]</td>
</tr>
<tr>
<td>Radiation necrosis</td>
<td>In some cases produces a peak at 2.4 ppm [65]</td>
</tr>
</tbody>
</table>

Table 3. Summary of $^1$H MRS characteristics of different brain tumours

Figure 3. Comparison of lesion extent measured using $T_2$ weighted MRI (red), $T_1$ weighted MRI following injection of contrast agent (green), and MRS to measure high Cho/NAA (orange) for a patient with a grade 4 glioma. Published with permission [43].

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reduction in Cho [42] and in lipid and lactate [43] can also reflect response to chemotherapy and radiotherapy.

MRS has been compared with MRI and with 201TlCl-SPECT (a radio-nuclide technique that is widely used to assess brain tumours) in monitoring response to treatment in metastatic brain tumours. Reductions in Cho and Lac and an increase in Lip (believed to represent necrosis) in responding tumours were detected at an earlier time (1 week to 1 month) than contrast-enhanced MRI or 201TlCl [44]. A short term change in metabolites reflecting both pathology and normal brain tissues has been reported within 1 day of radiotherapy [45] although the significance of this is not clear, as a short term metabolic response to immediate damage delivered by radiotherapy may not translate into a therapeutic gain.

**Example 2: MRS and prostate**

**Introduction**

In the prostate, T2 weighted MRI has much superior contrast to CT, transrectal ultrasound and digital rectal examinations. However, the sensitivity and positive predictive value are still only of the order of 83% and 50%, respectively [6]. Several investigators are currently evaluating the use of 1H MRSI in the prostate to aid target definition.

The main spectral peaks observed in normal prostate (Figure 4, right) are those of the choline-containing compounds (Cho) at 3.2 ppm, creatine and phosphocreatine (3.02 ppm) and a large peak from citrate (2.6 ppm). This latter peak is tightly coupled, and the appearance depends on the echo time used to acquire the data [47]. As in other tissues, choline compounds are associated primarily with membrane synthesis, while creatine is involved in energy metabolism. Citrate is a product of normal epithelial cell metabolism in the prostate, where high levels of zinc inhibit the enzyme aconitase and hence prevent the oxidation of citrate in the Krebs cycle that occurs in other cells. It is regulated by testosterone and prolactin [48]. Sometimes an additional peak may be detected between the choline and creatine peaks that arises from spermine, a polyamine characteristic of healthy prostate and which is also under androgen control.

In prostate cancer (Figure 4, left) choline is elevated and the normal production of citrate is reduced. In contrast, benign prostatic hyperplasia, an enlargement of the prostate commonly found in older men, is characterized by high levels of citrate. Hence the choline/citrate ratio is a fairly reliable measure of the presence of cancer. Lipids are also sometimes seen in cancerous tissues, although the significance of these is yet to be established.

1H MRSI data may be acquired from the prostate using an external phased-array coil. However, the best signal-to-noise ratio is achieved using an endorectal coil. This is usually well tolerated by patients. The main disadvantage of the endorectal approach is slight deformation of the prostate which needs to be allowed for in using the images for radiotherapy treatment planning. One study suggests that rigid endorectal coils are less of a problem in this respect than the more usual balloon coils [49]. Buscopan is often used to reduce involuntary motion. While many studies [50, 51] have used slice-localized 2D-MRSI, full 3D-MRSI is much preferred to obtain data from the whole of the prostate. Currently, the state-of-the-art at 1.5 T is to achieve voxels with a 3D isotropic resolution of 6.25 mm in an acquisition time of 17 min [52]. Studies have shown that owing to haemorrhage there is some degradation of in vivo spectra in the 8 weeks following transrectal biopsy (18.5% of peripheral zone voxels have been reported as degraded within 8 weeks of biopsy, and 7% for those examined more than 8 weeks after biopsy [53]).

**Validation of MRS in prostate for target definition**

A strong correlation has been found between negative MRSI and negative biopsy findings, and between positive MRSI and positive biopsy findings [54]. However, there is only a weak correlation between the concentration of prostate specific antigen (PSA, the current “gold standard”) and either biopsy or MRSI findings [54]. Step-section pathological examination of radical prostatectomy specimens demonstrated that MRI...
combined with MRSI yielded a significant improvement in cancer localization to a prostate sextant (left or right; base, mid-gland or apex) compared with MRI alone [55]. Several studies have shown that adding MRSI to an MRI examination increases the accuracy of diagnosis [55–57]. One particular area of high current interest is in discriminating the many patients who present with elevated PSA, but who have pathologically indolent cancer from those with aggressive disease; preliminary studies suggest MRSI has a useful role to play here as well [52]. High-resolution studies of tissue samples using MAS support these findings, with linear correlations measured between metabolite levels characteristic of normal epithelium or of prostate cancer, and the proportions of the corresponding cells as measured by computer-aided image analysis of prostate pathology slides [58].

Recurrence
Use of MRSI together with MRI has been shown to improve substantially the identification of tumour recurrence following external beam radiotherapy (the area under the ROC curve, a measure of the effectiveness of a test, increased from 0.5 to 0.81 [59]). The presence of 3 or more suspicious voxels in a hemi-prostate showed a sensitivity and specificity of 89% and 82%, respectively, for the diagnosis of local recurrence.

Planning
MRPI has been used in combination with MRI to define regions for dose escalation within the prostate [60–62], permitting a dose of > 90 Gy to the high-risk region while treating the remainder of the prostate to about 70 Gy.

Discussion
MRI already plays a major role in identifying the extent and position of tumours, aiding delineation of target volumes. Increasingly, there is interest in defining the functional extent of tumours to better select target volumes, or areas that might receive a boost dose. MRI already provides the capability to image several characteristics of tissues which reflect the physiology and microenvironment, such as perfusion and diffusion. MRPI provides the ability to observe aspects of tissue metabolism, which is a more direct reflection of tumour activity and of therapeutic response. The ability to obtain this information at the same examination as the anatomical and functional MRI images is a major advantage compared with other techniques. While the number of studies so far is limited, there is strong evidence that MRPI may have a valuable role to play in radiotherapy treatment planning.

The metabolic information provided by MRPI provides signals associated principally with tumour (elevated Cho, Lac, Lip) and with normal tissue (NAA, Cre, Cit), where Lip is likely to be associated with necrosis, allowing identification of different tissue components, and providing measures that may reflect tumour grade, normal tissue response, and areas of mixed tumour and normal tissue. The mismatch between metabolic information, function and anatomical abnormality is not surprising. In brain tumours, contrast enhancement depends on breakdown of the blood–brain barrier, which is not apparent in many low grade gliomas, and may not be present in early areas of infiltrative disease. Thus metabolism may be a better guide to early disease and disease extent compared with contrast enhancement. High signal on T2 weighted images reflects increased water content or local oedema, which may reflect inflammatory processes. Again, metabolic change could occur sooner, depending on the process controlling increased water content.

The main limitation with MRS is that voxel sizes of typically 8 mm3 to 10 mm3 are required to achieve an adequate signal-to-noise ratio, with a large part of this volume needing to be occupied by abnormal tissue for a change in signal to be detected. Thus, small infiltrating lesions are unlikely to be detectable. It also needs to be remembered that the edges of target volumes defined from spectroscopic images for treatment planning, although smoothed and interpolated for presentation, are in fact limited by this same spatial resolution and will also include some blurring from point spread function effects. While some improvement in sensitivity and spectral specificity is expected with higher field scanners and improved sensitivity coils, this is likely to yield only a small improvement in spatial resolution. On the other hand, it is increasingly clear that while in principle both MRI and CT have much better spatial resolution, MRS, has the potential to improve identification of the gross tumour volume and hence improve treatment using radiotherapy.

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References
MR spectroscopy and radiotherapy treatment planning


