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Pediatric Brain Tumors: Magnetic Resonance Spectroscopic Imaging

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1. Introduction

The incidence of cancer in the United States in children under 15 years of age has risen in recent years (Ries et al., 1991). This is largely due to the increased incidence of lymphoblastic leukemia and tumors of the brain and nervous system, as opposed to Wilms tumors, soft tissue and bone sarcomas, lymphomas and Hodgkin's disease or other malignancies of childhood. Between 1973 and 1988, the incidence of childhood nervous system tumors jumped by 30% (Bleyer, 1993). Every year, more than 1,500 children are diagnosed with brain tumors (Pollack, 1994). Because a child is more likely to develop cancer during the first 5 years of life, the etiology of these early cancers is likely different from those later in life and of different factors. While childhood tumors are more aggressive, their long-term control is often possible (Albright, 1993). Cancer Statistics Review reports an overall decrease in childhood cancer mortality, although brain and nervous system cancer deaths have decreased less than those due to other malignancies (Ries et al., 1991). These results indicate the need for useful *in vivo* biomarkers to allow the evaluation of treatment protocols for pediatric brain tumors. Given the inherent difficulties of sequential biopsies to monitor therapeutic response in children with brain tumors, non-invasive and non-irradiating imaging methods are needed to provide additional diagnostic indices or biomarkers beyond simple tumor volume measurements. Brain tumor treatment in most modern centers is managed through a tumor board, which typically rely in part on available proton Magnetic Resonance Spectroscopic Imaging (MRSI) results, especially for inoperable tumors that can be difficult to biopsy. Additionally, where progression or treatment response is questioned, serial *in vivo* MRSI is preferred over serial biopsy or PET/SPECT, which is irradiating, expensive and often unavailable. Non-invasive and non-irradiating *in vivo* MRSI can be performed as an adjunct to Magnetic Resonance Imaging (MRI), and is thus cost effective and the method of choice in children under 5 years, when radiation is a serious concern. Although MRSI does not obviate the utility of biopsy, it is suggested that it has the potential to replace serial biopsy and is an excellent alternative to biopsy in inoperable or unbiopsied tumors. The MRSI data when combined with anatomical or other type MR images provide unique information regarding brain tumor biochemistry in inoperable tumors and, might complement neuropathology, guide biopsies, and monitor therapy for operable brain tumors. The combination of such non-invasively acquired prognostic information and the high-resolution anatomical imaging provided by

conventional MR imaging will surpass molecular analysis or DNA microarray gene profiling, which although promising depend on invasive biopsy.

2. Application of brain tumor proton MRSI in children

Localized MR spectroscopy studies in children are increasing (Tzika et al., 1993a; Lazareff et al., 1996; Tzika et al., 1997; Lazareff et al., 1998; Lazareff et al., 1999; Warren et al., 2000; Tzika et al., 2001; Tzika et al., 2002; Tzika et al., 2003; Astrakas et al., 2004; Tzika et al., 2004). Brain tumors are usually heterogeneous and complicated by edema and necrosis of the adjacent brain parenchyma, yet their spectra are critically impacted by precision in voxel size and position. Single-voxel MR spectroscopy has the inherent disadvantage that spectral data is not simultaneously collected from the tumor and its surrounding tissue, which greatly hinders the incorporation of valuable information in assessing therapeutic response (Kurhanewicz et al., 1996; Nelson et al., 1997a; Wald et al., 1997; Dillon and Nelson, 1999; Nelson, 2001). Due to technical difficulties, a limited number of adult brain tumor studies using advanced localized MR spectroscopy have been reported (Wald et al., 1995; Nelson et al., 1997a; Nelson et al., 1997b; Wald et al., 1997; Graves et al., 2001a; Vigneron et al., 2001a; Li et al., 2002), and even fewer studies have been carried out in children, employing advanced localized MRSI (Lazareff et al., 1996; Tzika et al., 1997; Lazareff et al., 1998; Taylor et al., 1998; Gonen et al., 1999; Lazareff et al., 1999; Tzika et al., 2001; Tzika et al., 2002; Tzika et al., 2003; Astrakas et al., 2004; Tzika et al., 2004).

3. Biological aspects of selected metabolites detected by proton MRSI

Proton MR spectroscopy has identified several metabolites that are biomarkers of tumor growth and apoptosis (Tzika et al., 1997). To this end, brain tumor proton MR spectroscopy studies **(Fig. 1)** consistently demonstrate: (1) reduced or absent n-acetylaspartate (NAA) and total creatine (tCr) attributed to edema and necrosis; (2) increased Cho-containing compounds possibly due to cell membrane disruption (Griffin et al., 2001) and altered phospholipid metabolism (Aboagye and Bhujwalla, 1999; Podo, 1999; Ackerstaff et al., 2001); and (3) increased lactate due to metabolic acidosis (Bruhn et al., 1989; Alger et al., 1990; Arnold et al., 1990; Segebarth et al., 1990; Luyten et al., 1991; Nelson et al., 1997a; Nelson et al., 1997b; Wald et al., 1997; Aboagye et al., 1998). Reduced NAA is expected in glial tumors, since NAA is primarily localized to neurons. Therefore NAA detection within glial tumors corresponds to either partial volume averaging with adjacent normal tissue, or tumor infiltration of normal tissue. Since NAA occurs in cell cultures of oligodendroglia progenitors (Urenjak et al., 1992), NAA in childhood tumors may also reflect immature oligodendroglia. A reduction in tCr resonance may indicate cell loss due to necrosis, and correspond to exhausted energy reserves resulting from rapid cell proliferation and ischemia. It is also possible that tCr may be a valuable independent predictor of tumor response to therapy (Tzika et al., 2001).

The Cho peak consists of water-soluble Cho-containing compounds, such as phosphocholine (PCho) and glycerophosphocholine (GPC), and free choline (Barker et al., 1994), versus membrane-bound phospatidylcholine (Miller et al., 1996a). *In vivo* MRS reveals that phosphomonoesters (PME), such as phosphocholine (PCho) and phosphoethanolamine (PEth), are elevated in tumors and rapidly proliferating tissues (Daly et al., 1987; Daly and Cohen, 1989; Gillies et al., 1994a). Furthermore, elevations in PCho and PEth correlate with

increased cell growth or increased cell degradation, and have been shown to occur in human tumors, as well as in animal tumor models and cell lines. For instance, actively proliferating cultures show dramatically lower PCho/PEth as compared to stationary cultures (Aiken and Gillies, 1996). Gillies et al., found that PCho levels are lowest, and PEth levels are highest in non-proliferating cells and concluded there is a decrease in the biosynthesis of PCho concomitant with a reduction in culture growth (Gillies et al., 1994b). Mahmood et al., found a strong radiation dose-dependent response in the relative PCho/PEth ratio, suggesting that changes in the PCho levels may be related to cell proliferation and/or radiation-induced membrane damage (Mahmood et al., 1994). Aiken et al., suggested that growth stimulation in rat-2 fibroblasts increases phosphomonoesters suggesting that growth stimulation increases PCho levels (Aiken et al., 1996). Together, these data suggest the hypothesis that PCho, which can be measured with either phosphorous or proton MRS, is elevated in actively proliferating cells. Indeed, *in vivo* proton MRS studies suggest the Cho peak reflects proliferative activity in gliomas (Shimizu et al., 2000; Tamiya et al., 2000). Furthermore, PCho concentration correlates with the number of S-phase cells, with depletion corresponding to growth arrest (Smith et al., 1991), and the PCho/GPC ratio corresponding to oncogenic transformation (Bhakoo et al., 1996). Also, the PCho-produced Cho signal has been proposed to also depend on local cellularity (Chang et al., 1995; Miller et al., 1996b). Recently, using a high-resolution magic angle spinning proton MRS technique, it has been shown that PCho levels in glioblastoma multiforme correlate to the percentage of highly cellular malignant glioma (Cheng et al., 2000b). Also, altered phospholipid metabolism, such as PCho and GPC accumulation, has been reported to reflect early stages of growth arrest or apoptosis (Smith et al., 1991). In addition, GPC levels were found to increase in cultured mammalian cells exhibiting perturbed energetic metabolism during acidosis (Galons et al., 1995). In general, the consensus is that tissues with high proliferative potential or tissues that are oncogenically transformed are also highly cellular in the absence of compensating apoptotic mechanisms or limitations of vascular supply. Consistent with this, many studies strongly have suggested that the Cho peak detected by *in vivo* MRS may be elevated because the volume of interest is highly cellular (Chang et al., 1995; Miller et al., 1996b; Cheng et al., 2000b) or includes cells with high PCho which may be due to increased proliferative potential (Daly et al., 1987; Daly and Cohen, 1989; Gillies et al., 1994a; Mahmood et al., 1994; Aiken and Gillies, 1996; Aiken et al., 1996) or includes cells which are oncogenically transformed (Bhakoo et al., 1996; Aboagye and Bhujwalla, 1999; Ackerstaff et al., 2001). It was recently reported that in *ex vivo* high resolution MR spectra (which show much higher than the spectra obtained *in vivo*) of a biopsy from a cerebellar primitive neuroectodermal tumor, myo-inositol, taurine and phosphorylethanolamine contribute to the *in vivo* signal at 3.2 ppm, usually attributed to Cho-containing compounds (Tugnoli et al., 2001).

Cancer cells are apoptotic, and thus typically die upon conventional chemotherapy, radiation (Thompson, 1995) and experimental approaches such as antiangiogenic (Holmgren et al., 1995) and ganciclovir treatments (Freeman et al., 1993; Wei et al., 1998). Recently, it was shown that *in vivo* proton MR spectroscopy detects a substantial accumulation of polyunsaturated fatty acids associated with gene therapy-induced apoptosis (Hakumaki et al., 1999). Furthermore, PCho depletion, a major constituent of the Cho peak detected *in vivo* in tumors, coincides with growth arrest (Smith et al., 1991). Therefore, facilitation of apoptosis by selective chemotherapeutic agents or gene therapy

could be a future strategy for human cancer treatment (Thompson, 1995). It has also been

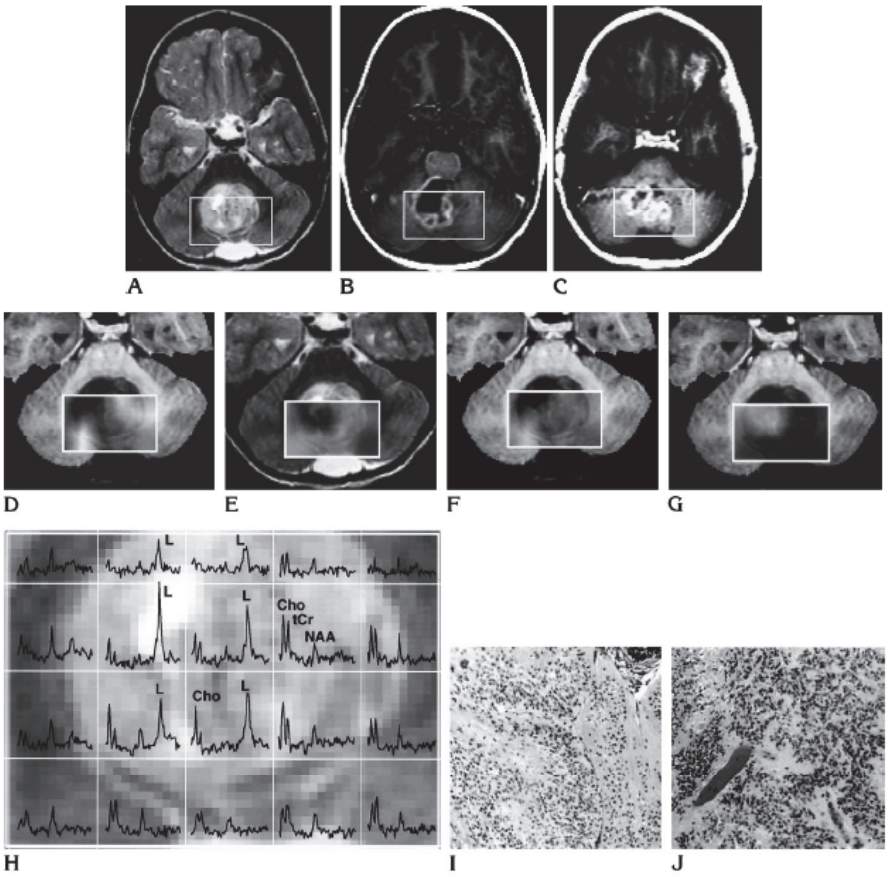

Fig. 1. MR imaging and MR spectroscopy in a 4-year-old boy with a large posterior fossa anaplastic ependymoma. *A*, Axial T2-weighted MR image shows a large cystic posterior fossa tumor. *B* and *C*, Two contiguous axial T1-weighted MR sections after injection of contrast material show a large cyst and nonhomogeneous enhancement. *Rectangles* indicate the volume selected for MR spectroscopy. *D–G*, Composite images represent Cho (*D*), NAA (*E*), tCr (*F*), and lactate/lipids (*G*) metabolite distributions (*rectangles*) superimposed on the T2-weighted MR images. Increased brightness corresponds to higher metabolite levels and decreased brightness to lower levels. In *D*, *F*, and *G*, the T2-weighted image contrast has been reversed so that the tumor is dark and does not interfere with the intensity of the metabolite image. Note that the cyst (clearly seen as a dark region in *B*) corresponds to low or no NAA and high lactate/lipids, which suggests that lactic acid has been concentrated in the cyst. *D* shows two regions of high Cho corresponding to the solid portion of the tumor. *H*, The spectral grid has been superimposed on a zoomed T2-weighted image. The metabolic heterogeneity of the tumor as depicted by multivoxel MR spectroscopy is illustrated. *I* and *J*, Histologic sections show certain regions of the tumor are "typical" ependymoma (*I*), whereas other regions had more anaplastic features, including increased cellularity (*J*).

inferred that prior to volume loss, the treatment response is associated with an increase in tissue water diffusion and T2 relaxation time (Poptani et al., 1998), which suggests increased water content and bulk diffusibility. Also, reduced diffusion of Cho-containing compounds in gliomas undergoing apoptosis has been reported (Hakumaki et al., 1998). These observations imply an increased viscosity and restriction within cells, to reflect cell shrinkage. Also, flow-cytometric studies demonstrate that gene therapy-induced apoptosis (Freeman et al., 1993) is preceded by an irreversible arrest in the late S- or G2-plase of the cell cycle (Wei et al., 1998). The MRS-visible lipids not only correlate with apoptosis or necrosis (Cheng et al., 2000a; Tugnoli et al., 2001), but also with the proportion of cells in these S- or G2 stages (Veale et al., 1997). Finally, the ceramide resonance region has been associated with the differential diagnosis of high and low malignancy of brain gliomas (Lombardi et al., 1997). This observation deserves further investigation, since apoptotic stimuli such as ceramide, a second messenger related to apoptosis, disrupt electron transport in mitochondria (Kyriakis and Avruch, 1996; Susin et al., 1997; Kolesnick and Kronke, 1998; Schwandner et al., 1998; Williams et al., 1998; Yasuhara et al., 1999), which acts as an important site for apoptosis initiation (Ashkenazi and Dixit, 1998; De Laurenzi and Melino, 2000; Tournier et al., 2000).

4. Methodology for acquiring proton MRSI data in the clinical setting

It is important to note that the methodological aspects of MRSI are not standardized and may vary among investigators or clinical sites. Typically, for MRSI, a large volume of interest can be selected and then phase encoding is applied to obtain multiple voxels in a single plane (Tzika et al., 1997) or in three-dimensions (Nelson et al., 1997c; Vigneron et al., 2001b). The advantage of obtaining multivoxel data is that it is possible to observe not only heterogeneity within the lesion but to examine surrounding tissue that may appear normal on MR images. This provides a reference for comparing metabolite levels in the tumor and makes it possible to identify regions of abnormal metabolism outside the morphological lesion (Nelson et al., 1999; Graves et al., 2000; Graves et al., 2001a; Tzika et al., 2002). According to our experience with MRSI in children, multilevel two-dimensional MR spectroscopy data acquisitions with no gap may be rather used than three-dimensional methods. This approach improves the signal-to-noise ratio, because adjustments for magnetic field homogeneity and water suppression may be performed in each section; with a large volume, these adjustments often fail in the clinical MR setting (Tzika et al., 2002). The two most common methods used for volume pre-selection is point-resolved spectroscopy (PRESS) (Bottomley, 1984; Bottomley, 1987) or stimulated echo acquisition mode (STEAM) (Bruhn et al., 1989), with PRESS being preferred when the echo time allows because of its intrinsically higher signal to noise ratio (Tzika et al., 1996b). Briefly, after a 50–100-mL volume is selected and after shimming and water suppression adjustments are made, a large data set is obtained by using phase-encoding gradients in two or three directions. The following parameters may be used for 2 dimensional acquisitions: 1000/65 (TR/TE), 16 X 16 phase-encoding matrix, 160-mm FOV, section thickness of 10 mm, 1250-Hz spectral width, two averages, and 512 points. Using these parameters, data sets of 1–1.2-cm^3 resolution are acquired. The decision to use a TE of 65 milliseconds may be made for the following reasons. If one is not as interested in the lactic acid detection as in the presence of lipids, which might be important, because lipids are related to tumor necrosis or apoptosis; this, in turn, is a determining factor of tumor activity. Our current notion is that a TE of 65 milliseconds provides us with the opportunity to 1) null lactic acid; 2) increase sensitivity in

lipid detection, and 3) prevent diffusion artifacts and water-suppression failures with PRESS performed at TEs shorter than 65 milliseconds. Thus, the four prominent peaks of biologic importance in our studies were those of NAA, Cho, tCr, and lipids (and/or lactate). An advantage of the above stated approach is that the volume of interest can be selected to eliminate as much of the subcutaneous lipids as possible and to avoid regions likely to cause large variations in susceptibility such as the sinuses. This permits improved shimming and provides spectra with narrower peaks and higher signal to noise. **Fig. 1** shows examples of multivoxel spectra from normal brain tissue, necrosis, and different regions from brain tumors (Tzika et al., 1997). The normal brain has N-acetylaspartate that is approximately twice the intensity of choline and creatine. Tumor generally has decreased N-acetylaspartate and increased choline and variable levels of creatine. Peaks corresponding to lipid and/or lactate may be present in regions of necrosis (Tzika et al., 1997; Tzika et al., 2002).

MRSI is more demanding in magnetic field homogeneity than MRI. In many circumnstances (e.g. close to the sinus or to cavities of resected tumors, or close to permanent radioactive seeds) shimming fails and water and lipid suppression become inadequate compromising the quality of the data obtained. Also due to the low spatial resolution of MRSI (about 1-cm^3 per voxel at 1.5 T) the spectra may reveal a mixed metabolic profile of tumor, necrosis, and normal brain tissue. Finally MRSI sequences are time consuming because usually they lack the most rapid form of gradient spatial enconding, namely the frequency enconding performed by the readout gradient. Many new approaches have been developed to improve the performance of conventional MRSI (Nelson et al., 1997b). Special alternative radiofrequency pulses are able to provide improved spatial and frequency selection (Star-Lack et al., 1997a; Star-Lack et al., 1997b) and better volume selection can be achieved with spatially selective saturation bands (Tran et al., 2000). Multislice and multiple echo time techniques (Spielman et al., 1992; Duyn et al., 1993) can be used to acquire multivoxel MRSI data in a time efficient manner. Also a fast three dimension multivoxel MRSI can be achieved with echo planar methodology with good quality data (Posse et al., 1994; Adalsteinsson et al., 1995; Posse et al., 1995). In the future the development of a hybrid PRESS-echo planar spectroscopic imaging technique with spatially selective saturation bands may speed up MRSI and overcome its present limitations in water suppression, volume selection and susceptibility artifacts (Nelson, 2003).

5. Processing and analysis of proton MRS data in the clinical setting

The processing and analysis of the resulting proton MR spectra combines fourier transforms and apodization with automated methods of spectral processing to provide data that can be interpreted by visual inspection or quantified to generate maps of the spatial distribution of different metabolites (Nelson, 2001). Different MR system manufacturers offer different packages for proton MRS analysis. Typically, the data are transferred off-line to the remote Sun workstation, converted into a standard data format, fourier-transformed and phased using appropriate spectroscopic packages. To reliably and reproducibly quantify *in vivo* spectra, requires removal of baseline components, identification of peaks, and estimation of peak parameters, which can be accomplished using several different approaches (Barkhuijsen et al., 1985; Hore, 1985; Laue et al., 1985; Nelson and Brown, 1987; Spielman et al., 1988; Van der Veen et al., 1988; Derby et al., 1989; Nelson and Brown, 1989). Characteristics of the proton MR spectroscopy data that guide the choice of methodology are the larger number of spectra that need to be considered, and the need for whatever

method is chosen to be robust to differences in signal to noise and peak configurations corresponding to different tissue types. Additionally, more sophisticated fitting algorithms can be applied to spectra that have sufficient signal to noise for the optimization routines to be reliable (e.g., (Provencher, 1993)). The output of the analysis is a number of spatial maps of metabolite parameters that can be applied to identify regions of normal and abnormal metabolism.

We have used a software application we have written in IDL that employs the PIQUABLE algorithm, which has the advantages (Nelson and Brown, 1987; Nelson and Brown, 1989) of being automated, uses non-parametric methods for objective identification of peaks, and can remove broad baseline components. This algorithm has been tested using simulated data (Nelson and Brown, 1987; Nelson and Brown, 1989) and data from human volunteers and patients (Nelson et al., 1997a). We have calibrated our software with simulated spectra, and spectra from phantom and patients, to result in reliable and reproducible results, within the accuracy of random noise. Additional corrections for spatial variations in intensity caused by the data acquisition procedures may also be required if comparing relative intensities of metabolites such as choline, creatine, N-acetylaspartate, lactate, and lipid (Nelson, 2001). When the PIQUABLE algorithm fails, we use alternative quantitation algorithms (AMARES, HLSVD, etc) in the MRUI package.

Several approaches may be used to display the information from multivoxel MRSI data sets and to correlate the anatomy with spatial variations in metabolites, including: (1) A grid superimposed on the MR image and plotting of the corresponding array of spectra. This approach does not require quantification and can be quickly performed after data collection; and (2) Metabolite images formed from arrays of estimated peak parameters. The primary resonances of interest are NAA, Cho, tCr, as well as lipid resonances at: 0.9 ppm (methyl groups), 1.3 ppm (methylene groups and lactate); 2.8 ppm (bisallylic methylene fatty acids); and a resonance at 5.4 ppm which arises from vinyl protons and includes ceramide. In addition, other metabolites, such as glutamate, glutamine, γ-aminobutyric acid, scyllo-inositol, aspartate, taurine, N-acetylaspartylglutamate, glucose and branched amino acids, may be detected (Tkac et al., 2001; Di Costanzo et al., 2003). To visualize the spatial distributions that correspond to the metabolites of interest, gray-level images mapping the peak area of these metabolites may be obtained; (3) Color metabolite images overlaid on gray-level MR images. This aids the estimation of the anatomic correlation of the varying levels of color metabolite images; (4) Selected spectra may be extracted from the MRSI data sets to be correlated with data from other modalities or with the *ex vivo* high resolution magic angle spinning proton MR spectra of tumor biopsies that correspond to the same anatomic location.

6. Contribution of proton MRSI in clinical tumor grading

Whether proton MRSI is able to contribute to defining tumor type and grade remains an open question. Although elevated Cho and low NAA before therapy may be a reliable indicator of pediatric brain tumor malignancy (Sutton et al., 1992; Tzika et al., 1993b; Tzika, 1995; Byrd et al., 1996b; Tzika et al., 1996a; Tzika et al., 1997; Taylor et al., 1998; Tzika et al., 2004), the consensus is that proton MR spectroscopy results may not be used alone to classify tumors, as some studies have shown considerable overlap in proton MRSI results among different tumor types (Warren et al., 2000; Warren, 2004). In our experience, the different MR spectral patterns may suggest that proton MRSI can be used to distinguish at

least three different tissue compartments —normal, tumor, and necrosis— (Naidich, 1995; Tzika et al., 1997), whereas mixed MR spectral patterns were due to the known heterogeneity of tumors, as confirmed by the histopathologic features (Tzika et al., 2002). This notion is in agreement with other reports (Ott et al., 1993; Wald et al., 1997; Star-Lack et al., 1998; Li et al., 2002). MR spectral patterns with elevated Cho and lipid levels in the absence of NAA that are histologically verified to represent regions of active tumor with extensive areas of necrosis suggest that such MR spectral patterns contribute additional information that is not available with conventional MR imaging. Because glial tumors are graded according to their cellularity, proliferative activity, and degree of necrosis, Cho mapping (increased cellularity and proliferative activity), may contribute added value to MR neuroimaging in patients with brain tumors, especially when it is combined with lipid mapping (necrosis and/or apoptosis). Indeed, contrast-enhancing regions with high lipid levels and low or no Cho levels, as shown in 11 patients with malignant or inoperable tumors, have been suggested to represent areas of high neoplastic potential intermingled with microscopic necrosis; this finding was verified at biopsy (Tzika et al., 2002). Low-grade tumors not enhancing on the T1-weighted Gd-enhanced images exhibit prominent peaks corresponding to Cho; although tCr and NAA peaks are occasionally detected the absence of lipids and or lactate is demonstrated **(Fig 2).**

A successful classification of pediatric patients with posterior fossa tumors was published by Arle *et al.,* (Arle et al., 1997) using single voxel proton MR spectroscopy and a computer-based neural network. The network combined MR spectroscopy data (ratios of N-acetyl-aspartate, choline, and creatine) with 10 characteristics of tumor tissue obtained from MR images, as well as tumor size and the patient's age and sex, and improved diagnostic accuracy by identifing 95% of the tumors correctly. However, given the differences in spatial extent of tumors, the question arises as to whether the single voxel MR spectroscopy is able to contribute to defining tumor type and grade. To this end, an excellent classification of adult patients with brain tumors was reported by Preul *et al.,* (Preul et al., 1996) who used two-dimensional proton MRSI and a multivariate pattern recognition analysis of peaks corresponding to choline, creatine, N-acetylasparate, lactate, lipid, and alanine. Grade 2 gliomas tended to have low lactate and lipid, some N-acetylaspartate, and some creatine. Grade 3 gliomas tended to have low lactate and lipid, less N-acetylaspartate and creatine, with higher choline. Grade 4 gliomas tended toward high lactate and lipid, with very low N-acetylaspartate. Upon visual inspection of spectral patterns and metabolite levels in each class, it was clear that meningiomas were distinguished as they were the only lesions that had alanine. Although these results were very promising, there has not yet been a prospective study using the statistical classification that these authors derived. One of the complications in analyzing data obtained with a multivoxel data acquisition technique is in determining which spectrum to consider for each lesion. Suggestions that have been made include using the most abnormal voxel and the average of all voxels within the lesion. Both of these approaches involve a subjective decision that takes the anatomical appearance of the lesion into account. For example, does the lesion include the entire T2 abnormality or is it restricted to the enhancing volume. As seen in the study by Li *et al.,* the spectral characteristics of these regions may be quite different (Li et al., 2002). The same issue is present with single voxel analysis, but in that case, the decision is made implicitly at the time of data acquisition by the choice of the selected volume. The studies from Dr. Nelson's group at UCSF have suggested that although it may be possible to detect mean differences between populations of gliomas with different grades based upon metabolite levels, there is

considerable overlap, both for mean metabolite levels or for the most abnormal voxels within the T2 lesion (Li et al., 2002).

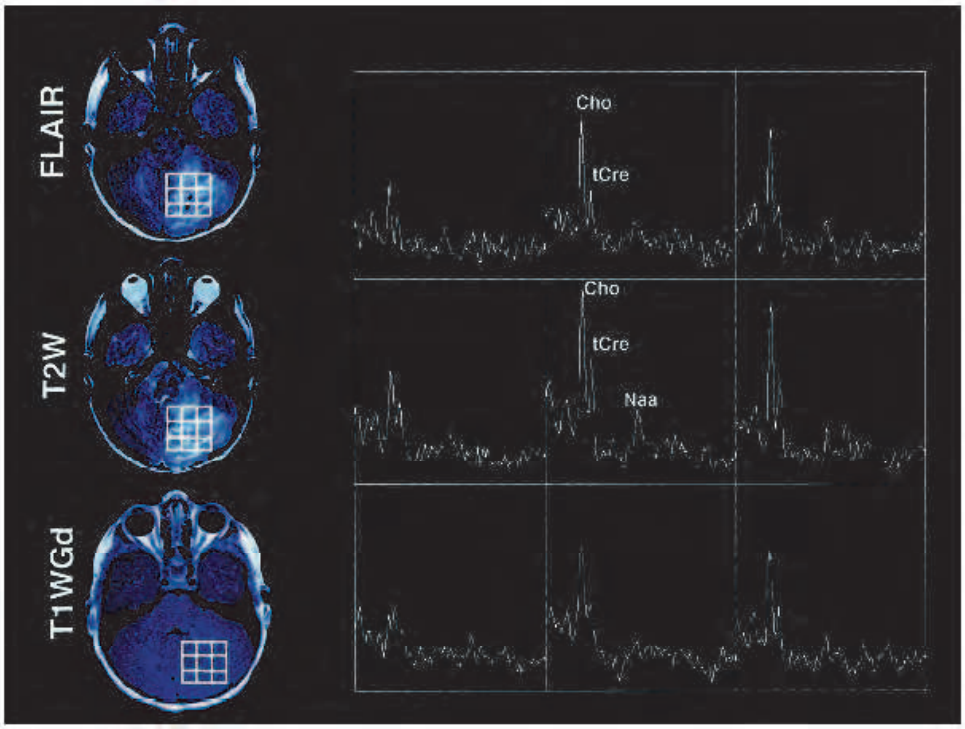

Fig. 2. Axial FLAIR, T2-weighted (T2W), and T1-weighted Gd-enhanced (T1WGd) MR images and selected proton MR spectra from a multivoxel MR spectroscopic data set in a 10-year-old girl with a cerebellar tumor. The lesion appears inhomogeneously hyperintense on the FLAIR and T2-weighted images and is not enhancing on the T1-weighted Gd-enhanced image. Prominent peaks corresponding to Cho are detected. Also, tCr and NAA peaks are occasionally detected. The Figure illustrates that no relationship existed between Cho detection and contrast enhancement on T1-weighted Gd-enhanced images and that spectral patterns devoid of lipid and/or lactate peaks are characteristic of low grade tumors. .

In our experience, information such as relative cerebral blood volume or apparent diffusion coefficient may also help in grading tumors and in distinguishing between tumors and other types of mass lesions (Tzika et al., 2003). Because brain tumors can be characterized according to their physiological parameters, including proton MR spectral metabolites (NAA, Cho, tCr, L), hemodynamic indices (i.e., rCBV) and physicochemical measures (ADC) the notion is that relationships among these parameters may reflect the biochemical state of tumors and this notion was supported by our findings so far (Tzika et al., 2003). Finally, age and tumor location in addition to anatomic factors seem likely to be relevant for classification and that which ever procedure is considered should ensure that the influence of all factors explicitly considered in the analysis.

7. Clinical role of proton MRSI in predicting response to therapy

A number of studies suggest that proton MRS spectroscopy promises an early prediction of whether a lesion has responded to therapy (Byrd et al., 1996a; Lazareff et al., 1998; Warren et al., 2000; Tzika et al., 2001; Tarnawski et al., 2002; Tzika et al., 2004). In a study involving 75 children with brain tumors, Byrd et al., found elevated Cho and elevated lactate and or lipids (Byrd et al., 1996a), which agrees with our data and data in adults. Another study involving 11 pediatric patients with low-grade gliomas, the tumors that progressed during a 2-year period displayed higher normalized Cho than those that remained stable (Lazareff et al., 1998). Warren et al., found in 27 children with recurrent or progressive tumors that the maximum tumor Cho:NAA ratio was predictive of outcome; Cho:NAA greater than 4.5 correpsonded to survival time of 22 weeks and all 13 patients died by 63 weeks; Cho:NAA less or equal to 4.5 corresponded to more than 50% survival at 63 weeks (Warren et al., 2000). Since proton MR spectroscopy detects total creatine (creatine, phosphocreatine; tCr) in addition to Cho and NAA in tumors, we hypothesized that we might be able to use proton MRSI to measure tCr to predict treatment response of pediatric brain tumors. To this end, 24 patients aged 10 months to 24 years were studied using MRI and point-resolved spectroscopy (PRESS) with volume preselection and phase encoding in 2-dimensions on a 1.5-T MR imaging system (TR-2000ms; TE-65ms). Multiple logistic regression was performed to establish the independent predictors of active tumor growth. Biologically vital cellular metabolites such as tCr, N-acetyl-aspartate (NAA), choline-containing compounds (Cho), and lipid or lactate (L), were seen to differ between tumor and control tissues ($P< 0.05$). Brain tumors (n=8), while responding to treatment (radiation or chemotherapy), exhibited decreased Cho ($P=0.05$), increased tCr ($P=0.02$), decreased NAA ($P=0.50$), and decreased L ($P=0.04$) when compared to untreated tumors (except surgery) or to tumors not responding to treatment (n=16), although the only significant independent predictor of active tumor growth was tCr ($P<0.01$). We concluded that Cho was the strongest metabolite signal detected in tumors, and tumor tCr was the only independent predictor of active tumor growth, and suggested that tCr is biological important metabolite useful in brain tumor assessment (Tzika et al., 2001). Other investigators have used other metabolite ratios such as lactate/NAA ratios to evaluate the prognostic value of MRS in brain tumors; for lactate/NAA ratios greater than 2.0 have been associated with 1-year survival rate of 20%, whereas for lactate/NAA values less than 2.0, the 1-year survival rate was 85% (Tarnawski et al., 2002).

In our experience, percent change in Cho/NAA is the most promising prognostic index in children with brain tumors (Tzika et al., 2004). From the serial proton MRSI exams of 27 children with neuroepithelial tumors we calculated and plotted the maximum percent change in Cho/NAA ratios versus clinical outcome. Each exam was rated either as stable or as progressive disease, according to the evaluation of the clinical oncologist who was blinded to the MRSI results. We used the Mann-Whitney U-test (since the Kolmogorov-Smirnov test detected significant skewness) and our results showed that percent change in Cho/NAA is significantly higher in the progressive ($n = 18$) as compared to the stable ($n = 32$) disease group ($P < 0.001$). Logistic regression confirmed that percent change in Cho/NAA is an important predictor of tumor progression (likelihood ratio test = 33.4, $P <0.001$). Using a 20% increase as a cutoff, Cho/NAA correctly classifies 16 out of 18

progressing cases (sensitivity = 0.89, 95% confidence limits = 0.65 – 0.99) and 27 out of 32 stable cases (specificity = 0.88, 95% confidence limits = 0.71 – 0.97). The odds of tumor progression are estimated to be approximately 55 times higher for cases showing at least a 20% change in Cho/NAA (odds ratio = 55.1, 95% confidence interval = 9.2 to 140.3). We also found significant differences between progressing and stable outcomes with respect to Cho/ntCr (progressing: median = 2.03, range 0.83 to 3.17; stable 1.53, range 0.74 to 6.81, P = 0.03). Furthermore, significant differences are seen between progressing and stable outcomes in percent change of Cho/ntCr (progressing: median +63%, range -14% to +140%; stable: median +9%, range -57% to +166%, P = 0.04).

For proton MRSI to be included in the clinical management of the patient it is important that MRSI improves the assessment of pediatric brain tumors by adding independent information regarding tumor involvement. For instance, if it were possible for proton MRS to assist in defining tumor borders since in gadolinium-enhanced MR images the relation between tumor cell extent and contrast-enhanced regions is unclear, it would allow modifying an ineffective treatment strategy before the tumor progresses further. To this end, we analyzed MRI and MRSI data in 31 children with brain tumors and we found that tumor spectral patterns were detected in tumor regions and outside enhancing tumor beds in patients with clinical progression; these were confirmed at neuropathologic analysis. This study demonstrates the importance of mapping out both the temporal and spatial distribution of metabolite changes in response to the therapy of interest. Such mapping requires the use of two or three-dimensional proton MRSI and is most easily achieved for the case of focal therapies such as surgery or radiation. The incorporation of multiple imaging modalities into therapy planning offers the potential to improve identification of regions of pathology. To this end, multiparametric and/or multimodality imaging has been proposed (Graves et al., 2001b; Tzika et al., 2003). Registration of the MR images and proton MRS data are critical for correlating data from such examinations. To this end excellent results from the studies in adults undergoing brain tumor therapy at the University of California San Francisco have been already reported (Wald et al., 1997; Dowling et al., 2001; Graves et al., 2001a; Graves et al., 2001b). In our opinion, multivoxel proton MRSI is more powerful than conventional or some of the recently promising types of MRI such as perfusion MRI for prediction of tumour behaviour (Tzika et al., 2004). Although proton MRSI generally agrees with perfusion MRI **(Fig. 3),** it may be superior to perfusion MRI in the case where perfusion MRI is limited **(Fig. 3).**

8. Clinical relevance of proton MRSI

The therapeutic approach to pediatric patients with malignant brain tumors is multifaceted and takes into account the location and resectability of the tumor, as well as the patient's age (Allen and Siffert, 1997). Surgery continues to be the treatment of choice for most patients, although overall effectiveness is often limited by disease dissemination or primary location (Mickle, 1997; Tomita, 1998). Radiation therapy has a documented role in the treatment of children with brain tumors (Buatti et al., 1997; Kalapurakal and Thomas, 1997), although most high-grade glial tumors show only temporary responses. Furthermore, the deleterious effects of radiation therapy on the developing nervous system often prevent the use of this modality. Finally, effective chemotherapy has been predominantly observed in neural

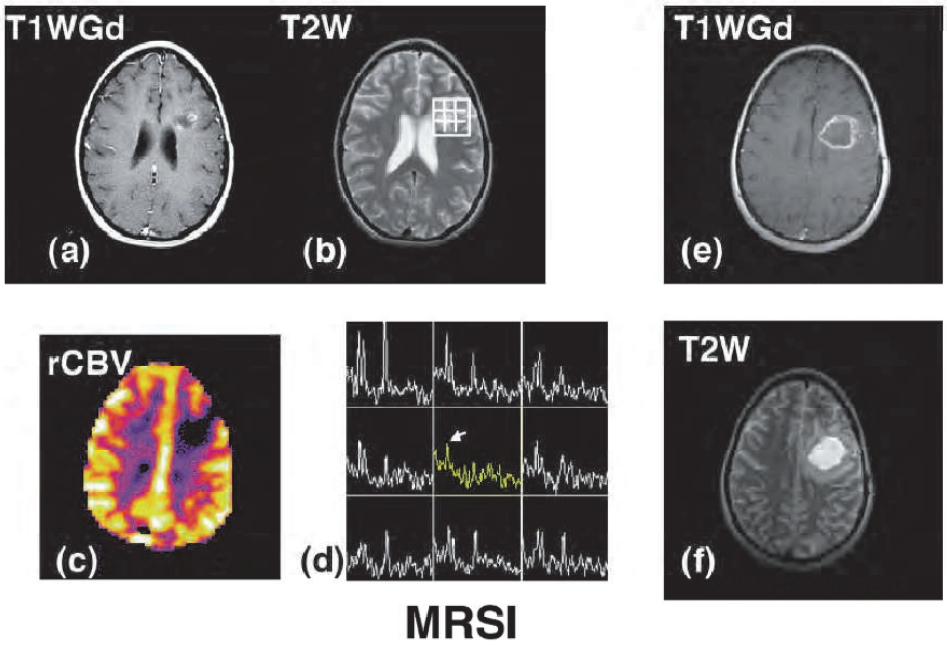

Fig. 3. Baseline magnetic resonance (MR) images and MR spectroscopic imaging (MRSI) (a–d) and 1-month follow-up MR images (e,f) from a female patient age 13 years with an anaplastic ependymoma during therapy. Gadolinium (Gd)-enhanced, T1-weighted (T1WGd) MR image (a) after Gd injection shows a region of enhancement within the left frontal lobe that is hyperintense on T2-weighted (T2W) image (b) and hypointense on a relative cerebral blood volume (rCBV) image (c). These findings may suggest tumor recurrence or radiation necrosis. The MRSI image (d) distinguishes radiation necrosis from tumor recurrence (middle voxel with a prominent choline [Cho] peak at arrow) and predicted tumor progression, which was evident on the T1WGd and T2W MR images (e,f) 1 month later. The rCBV image in c does not illustrate true relative tumor blood volume values, because the rCBV quantitation failed in the tumor region (most likely because of the leaky vasculature of the tumor), which is a limitation of the perfusion technique.

tumors and low-grade gliomas, but not in patients with high-grade glial or recurrent/progressive disease (Kedar, 1997). Given the difficulties inherent with sequential biopsy to monitor response to therapy in children with brain tumors, non-invasive and non-irradiating imaging methods are needed to provide additional diagnostic and/or prognostic indices or biomarkers beyond simple tumor volume measurements. Moreover, an important unresolved issue in brain tumor therapy is that dying or necrotic tissue within the CNS is difficult to differentiate from viable recurring tumor (Nelson et al., 1997a). By both clinical and standard CT or MRI scan criteria, necrosis or recurring tumor can appear to be identical. In time, tumor cell death will resolve, and thus the capability to differentiate between growing tumor and necrotic tissue at an early point in time is of great importance for both patient management and access to the biologic activity of tumorigenesis inhibitors or chemotherapeutic drugs. Advanced neuroimaging MR techniques, such as proton MRSI,

promise to help differentiate between these two entities at an early stage. Also, the spatial extent of the metabolic lesion by MRS is different from the gadolinium-enhancing region and hyperintensity on T2-weighted images (Tzika et al., 2002). Since there is such a distinction there may be added value for the proton MRSI data over and above conventional MRI. More importantly, the general consensus is that proton MRSI might be able to make an early prediction of whether a lesion has responded to therapy (Byrd et al., 1996a; Lazareff et al., 1998; Warren et al., 2000; Tzika et al., 2001; Tarnawski et al., 2002; Tzika et al., 2004). If this were possible, it would allow tailoring therapy to each individual patient and modifying an ineffective treatment strategy before the tumor progresses. It would also be possible to avoid giving unnecessary treatment in the case that an increase in tumor volume is attributable to treatment-induced necrosis as opposed to recurrent or residual tumor.

9. Conclusion

Although the clinical relevance of proton MRSI has not been decided yet, in our opinion, it is clear that proton MRSI improves the assessment of pediatric brain tumors by adding independent biochemical information regarding tumor type or grade, tumor involvement and by depicting residual or recurrent tumor outside the gadolinium-enhancing tumor bed. More importantly it is an invaluable adjunct to MRI and other modalities. To this end, it may provide biomarkers predicting tumor response earlier than conventional MRI. We believe that in the near future, and since higher field MR systems have been approved by the FDA and are being introduced in the clinical setting high-field, higher resolution proton MRSI, will provide unique biomarkers regarding brain tumor biochemistry in inoperable tumors and, might complement neuropathology, guide biopsies and monitor the success and failure of therapy, for operable brain tumors. Correlative studies with genomic biomarkers will strengthen the biological and clinical relevance of proton MRS.

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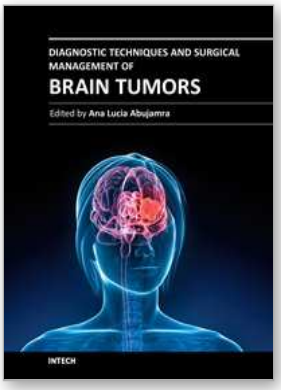

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The focus of the book Diagnostic Techniques and Surgical Management of Brain Tumors is on describing the established and newly-arising techniques to diagnose central nervous system tumors, with a special focus on neuroimaging, followed by a discussion on the neurosurgical guidelines and techniques to manage and treat this disease. Each chapter in the Diagnostic Techniques and Surgical Management of Brain Tumors is authored by international experts with extensive experience in the areas covered.

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