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Trends in Metabolical and Molecular Imaging of Glioma by Magnetic Resonance Spectroscopy

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Abstract: Magnetic Resonance Spectroscopy (MRS) is a non-invasive diagnostic and is the neuroimaging method of choice for the noninvasive monitoring of brain metabolism in patients with glioma tumors due to the enormous amount of information it yields regarding the morphologic features of the lesion and surrounding parenchyma. The most prevalent metabolites in the MRS spectrum are N-acetylasparate (NAA), total-Cholinecontaining metabolites (Cho), Lactate (Lac), Mobile Lipids (Lip), Creatine (Cre), Glutamate (Glu), Glutamine (Gln; the glutamate and glutamine signals cannot always be resolved and studies will then refer to their composite Glx peak), Myo-Inositol (mIns), Glycine (Gly), Glutathione (GSH) and 2-Hydroxyglutarate (2-HG). Personalized medicine using MRS can be used an understanding of the various physiological basic and mechanism of the metabolic signatures obtained from different types of tumors, and the specificity of the technique. Finally, establishment basis of physiological characteristics of the metabolites in various types of brain tumors, and the clinical utility of MRS as an additional and confirming diagnostic tool could improvement processes include fact and correct primary differential diagnosis therapeutic planning, and the assessment of response to treatment.

Key words: Metabolic imaging, magnetic resonance spectroscopy, brain tumor, glioma, neuroimaging

INTRODUCTION

Magnetic Resonance Spectroscopy (MRS) is a relatively fast, non-invasive method that provides metabolic biochemical information of the normal brain parenchyma and of pathological processes (Soares and Law, 2009). Spectroscopy can be done as part of a routine Magnetic Resonance Imaging (MRI) on commercially available MRI instruments (Ruiz-Cabello *et al*., 2011).

Magnetic resonance spectroscopy and MRI use different software to acquire and mathematically manipulate the signal. Whereas MRI creates an image, MRS creates a graph or "spectrum" arraying the types and quantity of chemicals in the brain or other organs (Hu *et al*., 2010; Golman *et al*., 2006). It is one of the methods for determining the molecular structures which provides metabolic information from viable brain tissues. Metabolites which were detected in brain tissue include Choline (Cho), Creatine (Cr), N-Acetylaspartate (NAA), lactate, Myo Inositol (MI), glutamine/glutamate, lipids and amino acids. Brain lesions contain abnormal quantities of these metabolites compared with normal brain tissue (Nekooei and Haratizadeh, 2014; Hammen *et al*., 2005; Ethofer *et al*., 2003). Many nuclei may be used to obtain MR spectra, including Phosphorus (31P), Fluorine (19F), Carbon (13C) and Sodium (23Na) (Solga *et al*., 2005; Holloway *et al*., 2011). The ones mostly used for clinical MRS are protons (H-MRS). The brain is ideally imaged with H-MRS because of its near lack of motion (this prevents MRS from being used in the abdomen and thorax without very sophisticated motion-reduction techniques) (Theberge *et al*., 2002; Cho *et al*., 2003). The hydrogen nucleus is abundant in human tissues (Keating and Knight, 2008). The H-MRS requires only standard Radio-Frequency (RF) coils and a dedicated software

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package. For non-proton MRS, RF coils tuned to the larmor frequency of other nuclei, matching preamplifiers, hybrids and broad-band power amplifier are needed (Weiss *et al*., 2005; Farzianpour *et al*., 2014). H-MRS is based on the chemical shift properties of the atom (Bertholdo *et al*., 2013; Deelchand *et al*., 2014). When a tissue is exposed to an external magnetic field its nuclei will resonate at a frequency (f) that is given by the larmor equation:

$$f = \gamma B0$$

There are different field strengths clinically used for conventional MRI, ranging from 0.2-3T. Since, the main objective of MRS is to detect weak signals from metabolites, a higher strength field is required (1.5T or more). Higher field strength units have the advantage of higher Signal-to-Noise Ratio (SNR), better resolution and shorter acquisition times making the technique useful in sick patients and others that cannot hold still for long periods of time (Di Costanzo *et al*., 2003; Abedi *et al*., 2012). MRS is applied in wide range of conditions such as evaluation of brain development, brain tumors and response rate to therapy, non-neoplastic brain lesions, epilepsy, Alzheimer's disease, degenerative and metabolic disorders, stroke, hypoxic-ischemic injury, Acquired Immune Deficiency Syndrome (AIDS), infections, multiple sclerosis and psychological disorders such as schizophrenia. Researchers have found that metabolic ratios obtained by MRS are useful in predicting malignancy and histological grade of brain tumor (Majos *et al*., 2003, Opstad *et al*., 2008). NMR spectroscopy, including in vivo Magnetic Resonance Spectroscopy (MRS) and high-resolution solution-state analysis of tissue extracts, have been widely used for several years to distinguish between different cell lines and tumor types.

Although, NMR spectroscopy detects only a fairly small number of metabolites, it can still be used to monitor the activity of many cellular activities because so many metabolic pathways are connected (Beckonert *et al*., 2010; Howe and Opstad, 2003). So changes detected in the metabolite can be used to follow several seemingly unrelated pathways. MRS has been used to analyze several tumor types in humans and in animal models of cancer and despite limitations in sensitivity and the ability to measure a broad range of metabolites, metabolomics profiles have been successfully used to distinguish between tumors types and between cell lines (Quintana *et al*., 2008; Griffin and Shockcor, 2004).

MRS can be valuable of the initial evaluation of brain tumors because of the different patterns of metabolites seen in tumors and because the abnormalities become more severe in advanced disease. Astrocytoma show a relative reduction in NAA and Cr while Cho increases relative to other metabolites in the progression from normal white matter to Grade 3 astrocytoma (Moreno-Torres *et al*., 2004).

In glioblastoma multiforme, some areas of the tumor may demonstrate a decrease in Cho, probably due to intratumoral necrosis (Liang *et al*., 2005). Abnormalities in the levels of alanine, lactate, and mobile lipids are also helpful in determining the grade of a glioma. The spectra of metastases are similar to those of astrocytomas and lymphomas with low NAA, low Cr and high Cho levels. However, one study has shown that the pattern of lipid/macromolecules signals differ in metastases and astrocytomas (Law *et al*., 2002; Vuori *et al*., 2004, Pirzkall *et al*., 2004).

MRSI can be very helpful in selecting a brain tumor biopsy site because the degree of metabolic abnormalities is more marked in areas with greater infiltration of cancer cells (Stadlbauer *et al*., 2004). In MSRI, data are gathered from several 1x1x1 cm voxels whereas single voxel MRS, the voxel size is typically 2×2×2 cm (Wright *et al*., 2009).

MRS is useful in follow-up imaging after treatment, where the differentiation between tumor recurrence and radiation necrosis is often difficult in conventional MRI. However, in some cases, the metabolic profiles are readily distinguished by MRS since a Cho/Cr ratio of >3 is associated with tumor progression and a ratio of <2 suggests tumor necrosis (Wagner *et al*., 2003). Gliomas are the most common primary tumors of the central nervous system. These tumors are classified into low-grade astrocytoma (grade 1/2), anaplastic astrocytoma (grade 3) and Glioblastoma Multiforme (GBM) (grade 4).

Noninvasive and accurate grading of brain gliomas is important for determining the correct treatment plan and in some cases to avoid unnecessary aggressive surgical treatment (Liimatainen *et al*., 2009; Zonari *et al*., 2007).

Proton Magnetic Resonance Spectroscopy (1H-MRS) is a technique used to obtain a biochemical profile of brain tissue and can provide biomarkers of neuronal integrity, cell proliferation or degradation, energy metabolism and necrotic transformation of tissues that complement the anatomical information available from conventional MRI. Multivoxel MRS (MRSI) provides high spatial coverage and may be more useful than single-voxel techniques for obtaining a metabolic map of a large size of tumors. Yet, Single-Voxel MRS (SVS) also has been reported to be useful for assessment of glioma grade.

Another important parameter that can largely influence the spectrum is the Echo Time (TE). At short TE, it is possible to detect more metabolites. However, there are several disadvantages such as the distortion of the spectra baseline under the effects of eddy current, water contamination and the overlapped lipids and lactate peaks, resulting in higher shimming demands (Majos *et al*., 2004). On the contrary, intermediate TE MRS may be chosen to detect the metabolites of longer relaxation times with little or no contamination of residual water, lipids or fat tissue and thus without baseline distortions. Few studies have been devoted to the contribution of both short and intermediate TE MRS for tumor grading.

MATERIALS AND METHODS

MRS and glioma: Proton MR Spectroscopy (MRS) analyses the biochemistry of a brain tumor and provides semi-quantitative information about major metabolites (Hammen *et al*., 2005; Ethofer *et al*., 2003; Solga *et al*., 2005). A common pattern in brain tumors is a decrease in N-Acetyl Aspartate (NAA), a neuron specific marker and Creatine (Cr) and an increase in Choline (Cho), Lactate (Lac), Lipids (L). The concentration of Cho is a reflection of the turnover of cell membranes (due to accelerated synthesis and destruction) and is more elevate in regions with a high neoplastic activity (Ethofer *et al*., 2003; Cho *et al*., 2003; Di Costanzo *et al*., 2003). Lactate (Lac) is the end product of non-oxidative glycolysis and a marker of hypoxia in tumor tissue. This is of increasing interest as tumor hypoxia is now recognized as a major promoter of tumor angiogenesis and invasion. Lac is probably associated with viable but hypoxic tissue, whereas mobile Lipids are thought to reflect tissue necrosis with breakdown of cell membranes (Solga *et al*., 2005; Holloway *et al*., 2011; Theberge *et al*., 2002; Cho *et al*., 2003; Keating and Knight, 2008; Weiss *et al*., 2005).

The choice of Echo Time (TE) is important technical considerations for performing MRS. It can be short (20-40 ms), intermediate (135-144 ms) or long (270-288 ms). MRS with a short TE has the advantage of demonstrating additional metabolites which may improve tumor characterization such as myo-Inositol, Glutamate/ glutamine (Glx) and lipids but is hampered by baseline distortion and artefactual NAA peaks. Intermediate echo times have a better defined baseline and quantification of NAA and Cho is more accurate and reproducible.

Long echo times lead to a decrease of signal to noise. MRS was performed using two different approaches have been implemented, namely, Single Voxel Spectroscopy (SVS) and Chemical Shift Imaging (CSI) (Ruiz-Cabello *et al*., 2011; Ethofer *et al*., 2003; Beckonert *et al*., 2010).

According to the first, a 3D area or volume of tissues is excited and the signals detected from this volume are transformed to a spectrum. In the second technique, multiple voxels are utilized either in a plane (2D CSI) or in a volume (3D CSI); therefore, it is possible to study larger areas with a single experiment (Howe and Opstad, 2003; Liang *et al*., 2005). Metabolic maps can be calculated based on the information derived from each voxel. SVS produces a single spectrum from a single voxel that is typically 8 cm^3 in volume whereas CSI measures spectra from multiple voxels that are typically 1–1.5 cm^3 in volume (Wright *et al*., 2009; Wagner *et al*., 2003). CSI data may be presented in a variety of displays including individual spectra, spectral maps or colored metabolite images overlaid on anatomical images. In MR spectroscopy, different Echo Time (TE) values can be utilized to control the "T2 contrast" of spectral peaks in the same way tissue T2 contrast is controlled in conventional imaging sequences (6-8, 13) (Hammen *et al*., 2005; Ethofer *et al*., 2003, Solga *et al*., 2005; Weiss *et al*., 2005). Metabolites with short T2 relaxation times decay faster and the corresponding spectral peaks are not seen on long TE spectra. This type of metabolites can only be detected on short TE acquisitions. The major healthy brain metabolite peaks that are seen on long TE spectra include N-Acetyl Aspartate (NAA) at 2.02 and 2.6 ppm which is a neuronal marker, Choline (Cho) at 3.20 ppm which is a membrane marker and Creatine (Cr) at 3.02 and 3.9 ppm which is an energy marker and generally is stable (Majos *et al*., 2003; Howe and Opstad, 2003; Griffin and Shockcor, 2004).

Short TE spectra contain additional peaks, which include Glutamine and glutamate (Glx) between 2.05-2.5 ppm and 3.65-3.8 ppm, Scyllo-inositol (sI) at 3.36 ppm, glucose at 3.43 and 3.8 ppm and myo-Inositol (mI) at 3.56 and 4.06 ppm which is a glial marker. The most important advantages of SVS over CSI are: shorter acquisition times, better localized shimming, simpler in terms of post-processing and precise volume definition (Bertholdo *et al*., 2013; Deelchand *et al*., 2014; Di Costanzo *et al*., 2003; Majos *et al*., 2003; Opstad *et al*., 2008; Beckonert *et al*., 2010).

The most important disadvantage is that SVS can provide spectra only from one voxel and it can be time consuming when multiple, remote areas should be evaluated. In many disease processes, biochemical changes are preceding morphologic alterations in tissues, therefore MR spectroscopy is a powerful technique to

identify early changes comparing to conventional MRI morphologic techniques. As a general rule, brain gliomas show increase of Cho and decrease of NAA peaks compared to normal brain tissue (Ethofer *et al*., 2003; Cho *et al*., 2003l; Deelchand *et al*., 2014; Law *et al*., 2002).

According to tumor grading the relative Cho/Cr and Cho/NAA ratios show significant increase from low to high-grade gliomas. The most important clinical applications of MR Spectroscopy either as a stand-alone technique or in combination with diffusion and perfusion weighted imaging techniques, can be summarized into the differentiation between low and high-grade gliomas, radiation induced necrosis and tumor recurrence, primary and secondary malignant tumors and abscesses and tumors (Howe and Opstad, 2003; Moreno-Torres *et al*., 2004; Pirzkall *et al*., 2004; Stadlbauer *et al*., 2004).

Another important clinical application of MR spectroscopy is the assessment of the therapeutic outcome by performing a baseline evaluation and follow-up experiments to identify therapeutic-induced changes and guide the therapeutic scheme. H-MRS examinations may be used to follow the progression of gliomas. Research shows that interval percentage changes in Cho intensity in stable gliomas and progressive gliomas (malignant degeneration or recurrent disease) is <35 and >45, respectively. Interval increased Cho/Cr or Cho/NAA is suggestive of malignant progression (Ethofer *et al*., 2003; Cho *et al*., 2003; Pirzkall *et al*., 2004; Stadlbauer *et al*., 2004).

RESULTS AND DISCUSSION

Grade II gliomas: H-MRS in low-grade gliomas may look similar to normal spectra, demonstrating a discrete reduction in the NAA peak, along with a discrete increase in the Cho peak. An increase in Myo can be the only finding in the spectra of a grade 2 astrocytoma. No lipids or lactate are usually demonstrated (Fig. 1). Low-grade glioma was studied *in vivo* at 4 T in 11 patients using H-MRS (incorporating the direct measurement of macromolecules in the spectrum) and Na imaging. The results showed that absolute levels of glutamate and NAA were significantly decreased whereas levels of Myo and 23Na were significantly increased in low-grade glioma tissue. The observation of decreased NAA levels is consistent with previous studies. The observed decrease in glutamate contradicts a previous study performed at 1.5 T that suggested that increased Glx maybe characteristic of low grade gliomas.

The discrepancy may be due to the removal of the macromolecule baseline signal intensity in1 the current study before quantification. The observed increase in Myo is consistent with previous studies.

Grade III gliomas: In grade 3 gliomas, there is a significant increase in the Cho peak which correlates well with high cell density in these tumors (Ethofer *et al*., 2003; Pirzkall *et al*., 2004; Wright *et al*., 2009). The NAA, Cr and Myo peaks are reduced. Metastases and glioblastomas nearly always show increased lipid peaks; thus if the

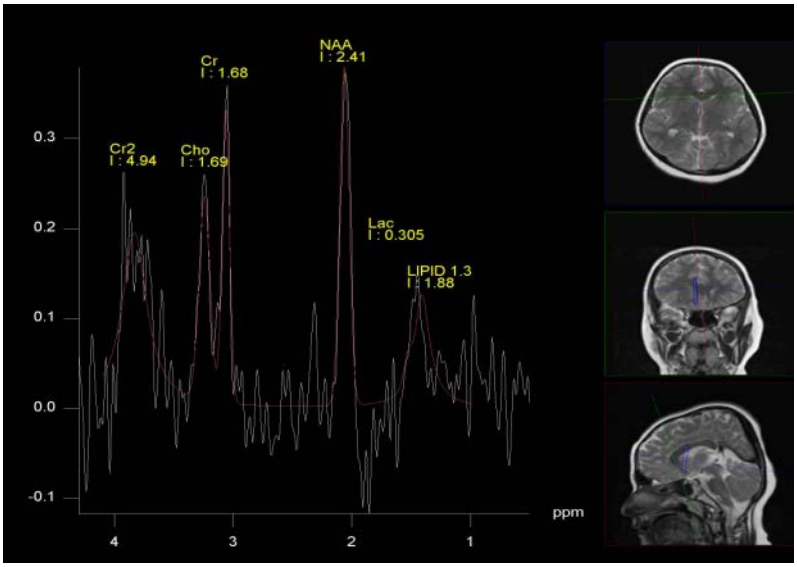

Fig. 1: Illustration of 47 male old years with gliomas which low-grade growths can be differentiated from high-grade types. Low-grade gliomas are generally characterized by a relatively high concentration of NAA as well as low level of Cho and absence of lactate

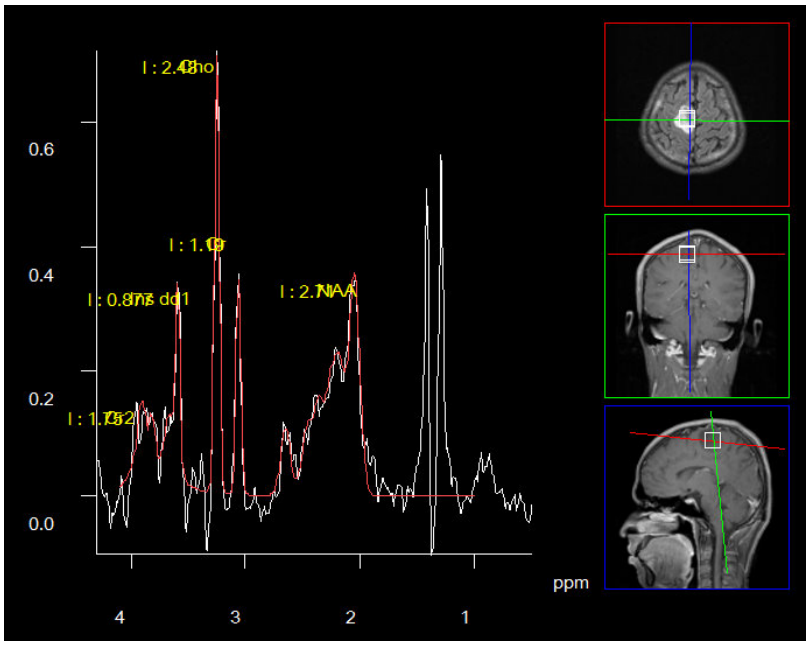

Fig. 2: Illustration of the 61 male old years with high-grade glioma that have higher mean Cho/NAA &Cho/Cr ratios than the low grade as well as reduction of NAA relative to Cr

lesion does not exhibit mobile lipid signals, anaplastic glioma is more likely. In the authors' experience, however, some increase in lipids and lactate may be seen in grade III gliomas (Ethofer *et al*., 200; Wright *et al*., 2009; Zonari *et al*., 2007; Majos *et al*., 2004).

Grade IV gliomas: The spectral pattern of grade IV gliomas is characterized by severe reduction of the NAA, Cr, and Myo peaks (Fig. 2). Cho is increased, although not as much as in a grade 3 glioma because a lot of necrosis is usually present in grade 4 gliomas which results in a significant increase in the lipid peak (Nekooei and Haratizadeh, 2014; Cho *et al*., 2003; Majos *et al*., 2003).

Typically, higher levels of Cho occur in grade 3 gliomas whereas, in GBM, the Cho levels may be much lower as a result of necrosis. When the voxel is placed within the necrotic area of a GBM, no Cho is detected and a prominent lipid lactate peak is the only spectral abnormality (Liang *et al*., 2005; Law *et al*., 2002; Vuori *et al*., 2004; Pirzkall *et al*., 2004; Stadlbauer *et al*., 2004).

CONCLUSION

H-MRS is a promising, noninvasive tool for predicting and monitoring the clinical response to chemotherapy drugs in patients with low-grade gliomas. Although there is considerable overlap between high-and low-grade tumor spectra, meticulously acquired spectra revealing choline/NAA ratios above 1.5 or analogous thresholds developed for choline/NAA ratios have been shown to improve the accuracy of anatomic MRI prediction of tumor grade. Because infiltration is a feature of glioma that cannot be detected reliably with current techniques, the significance of whole-brain NAA deserves further exploration as a marker of poor prognosis and diffuse tumor spread, but recently reported MRS technique for detection of tumor infiltration. A significant increase in choline plus a decrease in NAA over time, with a consequent increase in the choline/NAA ratio or in derived statistics such as the choline/NAA ratio R value, is a sensitive indicator of tumor recurrence when seen in the appropriate anatomic imaging context. We believed that using the Cho/NAA ratio correlated with cell density, cell proliferation index and the ratio of proliferating cells to dying cells, thus these techniques will become an essential clinical tool as a metabolic or molecular biomarkers for in the assessment of glioma response status.

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