Development of an MR spectroscopic index to differentiate tumor from treatment induced gliosis

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Introduction

The goal of this study was to identify MR spectroscopic (MRS) markers that are likely to be able to differentiate active tumor from treatment induced gliosis. This is an important problem because while tumor regions should be included in follow-up treatment plans, regions represented by gliosis should be left untouched since they represent areas of normal brain that are being influenced by treatment. Typically high MRS choline levels are used to indicate tumor presence. The challenge in differentiating tumor from gliosis using this metric is that both of them could result in elevated levels of choline. While the components that contribute to the choline peak may be different in tumor and gliosis, this is not evident from an *in-vivo* spectrum, which suffers from low spectral resolution. Hence proliferation and hypertrophy of astrocytes in response to surgical trama and various therapeutic modalities may be indistinguishable from tumor growth. To evaluate strategies for overcoming this ambiguity we use the following approach: 1) *ex-vivo* samples from the UCSF tissue bank that are confirmed as being tumor or gliosis with histopathology were studied using High Resolution Magic Angle Spectroscopy (HR-MAS) to identify specfic markers that differ between the two cases (non-image guided study), 2) the behavior of the same markers were investigated with *ex-vivo* HRMAS of biopsies obtained from within tumor regions from patients with newly diagnosed GBM with image guidance and 3) these markers were compared against known measures of tumor presence, choline-NAA index (CNI) [1] derived *in-vivo* from the 3T MRS voxel that contained the biopsy site. The high spectral resolution of the HR-MAS implies that it can reveal several differentiating markers between tumor and gliosis. Because the long-term goal of this study is to identify *in-vivo* markers we focused on metabolites that can be investigated with the 3T whole body scanner.

Methods

Ex-vivo acquisition and analysis: Samples were weighed and placed in custom designed 35 µl leak proof zirconium rotors. HR-MAS data were acquired at 11.7T, 1°C, and 2,250 Hz spin rate using a Varian INOVA spectrometer and a 4 mm gHX nanoprobe. Quantitative 1D spectra were acquired with the Carr-Purcell-Meiboom-Gill (CPMG) sequence; TR=4s; TE=144ms; 512 scans; 35 minute acquisition, 40000 points, 20000 Hz spectral width. The Electronic Reference to access in vivo concentrations (ERETIC) [2] method was used as a quantitation standard. Basis set spectra of 42 metabolites in solution and known macromolecular peaks were incorporated into a custom version of the QUEST [3] fitting routine. Concentrations were calculated relative to the peak area of the ERETIC signal. Results from 11 samples from tumor/gliosis non-image guided study and 6 biopsies (~2mm^3) from image-guided study are discussed here. In-vivo image guided analysis: Biopsy locations were recorded by Brain Lab software during the surgical procedure. These locations were then used to voxel-shift the 3T spectroscopic data so that the biopsy location was the center of the spectroscopic voxel. The 3T acquisition parameters were: TR/TE=1104/144 ms, 1 cm^3 resolution, 16x16x16 phase encoding matrix with flyback gradient [4] to reduce acquisition tme to ~ 9 minutes. The CNI associated with voxel containing the biopsy site was obtained using our custom analysis procedures [5].

Results and DIscussion

Table 1 shows the metabolite markers that were significantly different between tumor and gliosis in the non-image guided HR-MAS study. Despite the variability in these concentration measures, possibly due to heterogeneity in the origin of these samples, there is a statistically significant difference in Alanine, Creatine (Cre), Glycine (Gly), Myo-inositol (mI), Phospho-Ethanalomine (PE) and N-acetyl-aspartate (NAA). The total choline level i.e. the sum of Glycero-Phosphocholine (GPC) and Phospho-Choline (PC) was not different between the tumor and gliosis. NAA differences might be due to the origin of the gliosis samples from white/gray matter with epileptic pathology and not brain tumors. Given the notable difference in mI levels and that it is a known glial cell marker, we developed a Glial index (GI) as (= mI/[GPC+Pc]). The GI was found to be a singular marker that differentiated tumor and gliosis with high significance ($p < 0.001$). **Figure 1** illustrates these findings with a spectrum from confirmed 100% tumor (Black) and subpial astrogliosis (red). To evaluate the GI in the image guided study we acquired *ex-vivo* HR-MAS spectra (**Figure 2B**) from the biopsy locations (**Figure 2A: green cross**) and compared this spectrum to the 3T *in-vivo* spectrum (**Figure 2C**) from a voxel that contained the biopsy site. Figure 2B&C show a clear correspondence in the choline levels between the *ex-vivo* and *in-vivo* studies although given the poor resolution of 3T MRS the composition of elevated choline as originating from a GPC is not clear. Please note that since the *in-vivo* spectrum was acquired at a long TE = 144msec; metabolites such as myo-inositol were not measured due to their short T2 relaxation. A similar analysis was performed for all image guided biopsies and a significant negative correlation (r = -0.88) was observed between the glial Index (GI) for each biopsy and the CNI index obtained from the *in-vivo* MR data (**Figure 3**). Based on our non-image guided study higher values of glial Index are due to gliosis. This is consistent with lower tumor presence (low CNI) in these voxels.

Table 1	Tumor µ mol/kg (Mean ± sd)	Gliosis µ mol/kg (Mean ± sd)	T - test ($p < 0.05$)
Alanine	0.9 ± 0.6	0.5	0.01
Creatine	0.9 ± 0.5	1.6 ± 0.7	0.01
Glycine	1.7 ± 1.5	0.5 ± 0.1	0.04
mI	1.3 ± 0.8	3.0 ± 1.8	0.01
PE	1.2 ± 0.6	0.6 ± 0.2	0.03
NAA	0.1 ± 0.01	0.3 ± 0.1	< 0.001
Glial Index	1.2 ± 0.7	4.2 ± 1.3	< 0.001

Figure 1

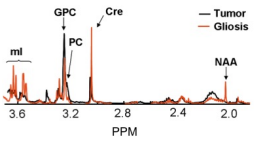

Figure 2

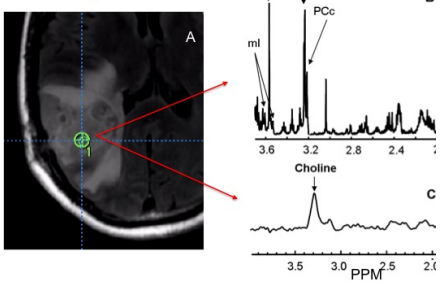

Figure 3

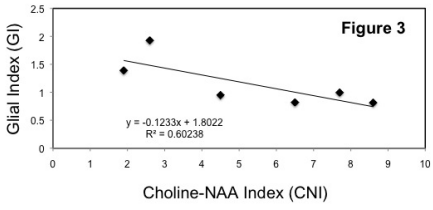

Table 1: Statistically significant MRS markers that differentiate tumor and gliosis in the *ex-vivo* HR-MAS non-image guided study **Figure 1**: Comparison of an *ex-vivo* spectrum obtained 100% tumor (Black) and gliosis (red). Levels of mI are significantly higher in gliosis. **Figure 2**: The *ex-vivo* spectrum (**2B)** from the biopsy location in (**2A**: green circle) compared with an *in-vivo* 3T MR spectrum (**2C**) from a voxel that contained the biopsy location. High choline levels are evident in both ex-vivo and *in-vivo* spectra. The CNI index of this voxel was 8.6 indicating the high tumor presence. **Figure 3**: Strong correlation (Spearman r = -0.88) between CNI and the Glial index in GBM patients. This finding, together with Table 1 and Figure 1 provide strong evidence that higher values of Glial Index are likely due to gliosis. Conversely lower Glial Index suggests tumor severity.

Conclusions

Here we develop a MR spectroscopic glial index in an attempt to differentiate tumor from gliosis. Future studies with more biopsies, associations of this index with additional MRI/MRS measures of tumor presence and *in-vivo* short echo MRS acquisitions with the ability to measure mI will provide further relevance to this measure.

References [1] Mcknight, et. al. J. Neurosurg, 2002 97(4):794 [2] Ziarelli et.al. SS NMR, 2006, 29:214 [3] Ratiney et al. (2005) NMR Biomed 18(1): 1. [4] Chen et. al. JMRI 2007, 25(6):1288 [5] Nelson MRM, 2001, 46(2): 228 **Acknowledgements:** NIH grants: RO1 CA59880 and PO1 CA11816-01A2