Changes in Serial Magnetic Resonance Spectroscopy Predict Outcome in High-grade Glioma During and After Postoperative Radiotherapy

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Abstract. *Aim: To determine any correlation between magnetic resonance spectroscopy (MRS) pattern of high-grade glioma before, during, and after radiotherapy (RT) with overall survival (OS) and progression-free survival (PFS). Patients and Methods: Twenty-six patients prospectively underwent surgery and RT to 60 Gy. MRS was performed before RT, at week 4 of RT, and 2 months post-RT. Normalized and relative metabolite ratios were evaluated. Patients were grouped according to similar evolving MRS patterns and analyzed for differences in OS and PFS. Results: Significant decreases in tumor choline/N-acetyl-aspartate and normalized choline were observed from baseline to post-RT. After a median follow-up of 22.9 months, patients with >40% decrease in normalized choline from week 4 during RT to 2 months post-RT had a significantly worse median OS (9.1 months vs. not reached, p<0.00001) and PFS (5.8 vs. 19.8 months, p=0.0018). Conclusion: The change in normalized choline at 2 months post-RT was highly prognostic for PFS and OS. This may allow more individualized response-based treatment.*

High grade gliomas (HGG) comprise the majority of malignant primary brain and central nervous system neoplasms (1). Standard therapy includes maximal resection and postoperative chemoradiotherapy. However, in patients with glioblastoma multiforme (GBM), prognosis remains poor despite multimodality therapy, with reported median overall survival (OS) of 14.6 months (2). One barrier to improving treatment of HGG is the difficulty in assessing early response to therapy.

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Currently, tumor response is evaluated with computed tomography or magnetic resonance imaging (MRI). However, such anatomic imaging techniques can be inaccurate as changes in contrast enhancing volumes may be due to tumor progression or treatment effect (3, 4). In addition, using decreasing tumor size as a surrogate for response in glioma may be inaccurate due to concurrent steroid usage, irregular and complex tumor shapes, and variations in imaging technique (5-7). Advanced MRI techniques, such as magnetic resonance spectroscopy (MRS), can evaluate cellular metabolism and may provide more information regarding response to treatment. MRS has already been shown to be capable of identifying active areas of tumor (8) and detecting tumor recurrence before changes in contrast enhancement are evident (9).

Proton MRS is a non-invasive MRI technique which provides qualitative and quantitative analysis of many metabolites such as N-acetyl-aspartate (NAA), choline, creatine, lactate, and lipids. NAA is a neuronal marker, and reduced levels are seen with most diseases of the central nervous system including glioma (10). The choline signal includes free choline, phosphocholine, and glycerophosphocholine. It reflects phospholipid metabolism and turnover of the cell membrane during rapid cell division or breakdown, and is elevated in most malignancies (10, 11). Creatine reflects the energy metabolism of the cell and decreases in hypermetabolic states (10).

To date, there has been little research investigating changes in MRS spectra during and soon after radiotherapy (RT), or the prognostic significance of temporal changes (12). This information would be valuable to individualize treatment by identifying non-responding patients for alternate therapy. Thus, we conducted a prospective trial investigating serial changes in MRS spectra during and after RT in patients with HGG and whether these changes predict treatment response and patient outcome.

Patients and Methods

Patient population. Patients with histologically confirmed HGG were prospectively enrolled from 2004-2006 at a single institution. Patients were eligible if they were ≥18 years of age and had adequate neurologic status to qualify for radical RT, as determined by the treating physician. Patients were excluded if they had prior RT to the head or neck, lupus, scleroderma, or contraindications to MR imaging. Each patient underwent surgical biopsy or resection, followed by postoperative 3D conformal RT. Tumor volumes were defined on computed tomography simulation with the aid of a diagnostic MRI. All patients underwent MRI and MRS imaging at baseline prior to RT, at week 4 during RT, and 2 months post-RT.

All MRS studies were performed on a 1.5 T clinical scanner (Philips Healthcare, Andover, MA, USA). We obtained T1- and T2-weighted images along with post-gadolinium single-slice multi-voxel MRS images. A point-resolved spectroscopic (PRESS) sequence was used for volume localization with TR=2200 ms and TE=272 ms. The size of the selected region (PRESS-box) was 10×11×2.0 cm with a 10×10×1 matrix. Each voxel measured 1.0×1.1×2.0 cm for an individual volume of 2.2 cm^3 (Figure 1). The PRESS-selected region was chosen to include the contrast-enhancing lesion, as well as contralateral normal tissue, while avoiding bone and subcutaneous fat which would complicate shimming and water suppression. Image acquisition was preceded by global and local shimming procedures. Spectra were obtained from two regions of interest. The first voxel was placed at the edge of the contrast-enhancing mass to cover as much of the imaging abnormality as possible, while the control voxel was placed in a corresponding area of the contralateral uninvolved brain (Figure 1). Care was taken to overlay the voxels as much as possible in the same site on each of the 3 longitudinal MRS studies.

The relative intensities of the signals from NAA (2.0 ppm), choline (3.2 ppm), and creatine (3.0 ppm) in the tumor and normal voxels were analyzed. The relative metabolite ratios and the normalized metabolite ratios (defined as tumor metabolite/contralateral brain metabolite) were calculated for each time point. The metabolite ratios examined included choline/NAA, choline/creatine, and NAA/creatine.

Statistical analysis. Patients were grouped based on similar evolving MRS patterns. Survival analysis was performed on patients who completed all three MRS scans. Patient survival was timed from the date of surgical diagnosis. OS was the duration to last follow-up or death. Progression-free survival (PFS) was the time to last follow-up or progression based on clinical or radiologic findings. Kaplan-Meier (KM) curves of OS and PFS were analyzed for statistical significance by the log-rank method. Paired groups of data examining changes in metabolite ratios over time for each patient were compared using the paired *t*-test. Results were considered significant at a value of $p<0.05$.

Results

Patient population. Twenty-six patients with HGG were enrolled with baseline characteristics listed in Table I. Of these patients, 18 completed the intended three MRS scans. Median follow-up was 22.9 months (range 5-37 months). Reasons for inability to complete all three MRS scans included patient deterioration (n=3), change in histology on pathology review (n=1), voluntary withdrawal (n=3), and poor spectral quality (n=1). At the time of analysis, 9 deaths had occurred and 6 patients were alive with progression. Concurrent chemotherapy consisted of either temozolomide for 12 patients or lomustine for 2 patients.

Table I. *Baseline characteristics of the patient population*.

Characteristic	All patients (n=26)
Age (years)	
≤50	10 (38%)
>50	16 (62%)
Gender	
Male	18 (69%)
Female	8 (31%)
Karnofsky performance score	
<70	3 (12%)
≥70	23 (88%)
Type of resection	
Biopsy only	5 (19%)
Subtotal or gross total resection	21 (81%)
Pathology	
Anaplastic astrocytoma	4 (15%)
Glioblastoma multiforme	17 (65%)
Anaplastic oligodendroglioma	3 (12%)
Mixed anaplastic oligoastrocytoma	2 (8%)
Concurrent chemotherapy during radiotherapy	
Yes	14 (54%)
No	12 (46%)
Time from surgery to first MRS (days)	
Median	41
Range	14-71
Time from surgery to start of radiotherapy (days)	
Median	44
Range	27-70
Number taking medications at time of MRS	
Steroids	23 (88%)
Anticonvulsants	19 (73%)
Number MRS scans completed	
1	26 (100%)
2	20 (77%)
3	18 (69%)

Analysis of metabolites. Comparisons of mean metabolite ratios for tumor and normal brain at baseline, 4 weeks of RT, and 2 months post-RT were made. Significant differences existed between the tumor and normal brain metabolite ratios for choline/NAA ($p=0.021$), choline/creatine ($p=0.020$), and NAA/creatine ($p=0.002$) for all three MRS scans at 2 months post-RT. Although there appeared to be a trend towards 'normalization' of the choline/NAA and choline/creatine ratios, with the absolute differences between tumor and normal brain becoming smaller over time, the differences continued to be significant even at 2 months post-RT.

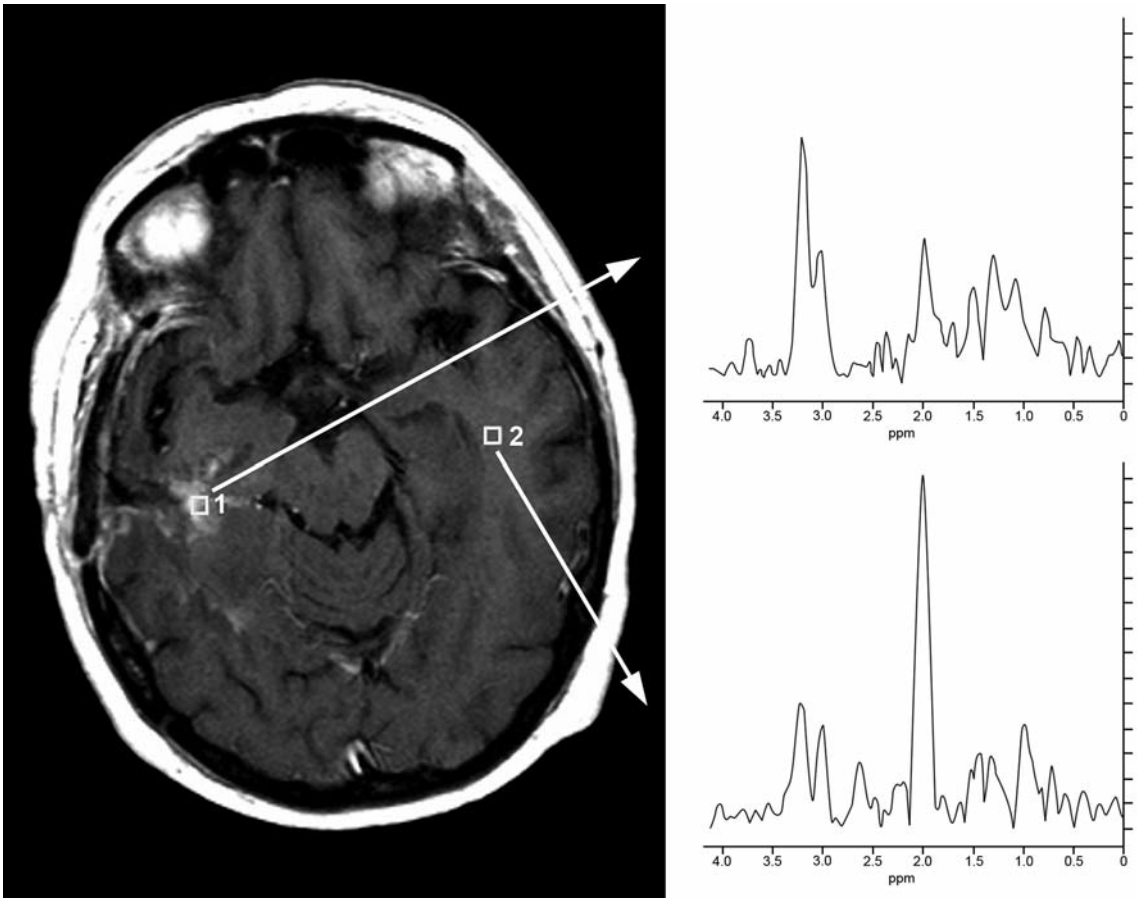

Figure 1. *MRS region of interest and individual voxel selection across serial images with corresponding MR spectra. Note that the square shown for voxel selection is centered in an individual voxel volume of 1.0×1.1×2.0 cm.*

The mean tumor choline/NAA ratio decreased after RT, with a significant difference between the baseline and post-RT scans (1.75±0.83 *vs*. 1.23±0.52, p=0.028). The tumor choline/creatine and NAA/creatine ratios were not significantly different over this time period.

Similarly, the temporal evolution of normal brain metabolite ratios showed significant differences between the scans at baseline and post-RT. There was an increase in the choline/NAA ratio between the baseline and post-RT scans (0.40±0.11 *vs*. 0.51±0.29, p=0.044), as well as from week 4 to post-RT (0.49±0.37 *vs*. 0.51±0.29, p=0.035). There was also a decrease in the NAA/creatine ratio from baseline to 2 months post-RT (3.46±0.68 *vs*. 2.98±0.95, p=0.048).

Temporal changes in the normalized ratios of choline, creatine, and NAA were analyzed. The normalized choline showed a significant decrease between baseline and post-RT (1.67 *vs*. 1.04, p<0.0001) as well as from week 4 to post-RT (1.45 *vs*. 1.04, p=0.04), as shown in Figure 2. The normalized creatine ratio also showed a decrease between the scans at baseline and 2 months post-RT (1.00 *vs*. 0.70, p=0.033). The normalized choline/NAA ratio decreased over the study period, with a significant difference found between baseline and post-RT scans (4.53 *vs*. 2.76, p=0.004).

Survival analysis. Median OS of the patients, who completed all three MRS, was not reached, and the median PFS was 13.1 months. The extent of resection was prognostic, with patients, who underwent subtotal or gross total resection (GTR) experiencing a superior median OS (27.1 *vs*. 6.7 months, p<0.0001) and PFS (16.0 *vs*. 4.7 months, p=0.0004) than patients with biopsy alone. Concurrent use of chemotherapy was also a favorable prognostic factor for PFS (21.3 *vs*. 6.6 months, p=0.0021) and showed a trend for an improvement in OS (28.3 *vs*. 16.0 months, p=0.0872).

Patients with >40% decrease in normalized choline between scans at week 4 and post-RT had a markedly worse median OS (9.1 months *vs*. not reached, p<0.00001) and PFS

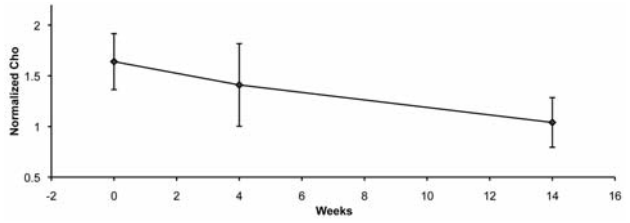

Figure 2. *Temporal changes in normalized choline (mean±95% confidence interval).*

(5.8 *vs.* 19.8 months, p=0.0018), as illustrated in Figures 3 and 4. However, no significant association was found when examining the change in normalized choline from baseline MRS to 2 months post-RT (p=0.11). Three out of the 18 patients who completed all three MRS scans underwent GTR, two of whom had a >40% decrease in normalized choline. On Fisher's exact test, the number of GTRs in each prognostic group was not statistically different (p=0.528). Sensitivity analysis excluding GTR patients shows the median OS was still worse for the group with >40% decrease in normalized choline (10.4 months *vs.* not reached, p=0.009). However the difference in PFS between groups lost significance (4.8 *vs.* 18.4 months, p=0.286). Analyzing only patients with GBM showed that patients with >40% decrease in normalized choline had a worse median OS (10.1 months *vs.* not reached, p=0.0047) although the median PFS was not significantly different (6.7 *vs.* 15.9 months, p=0.28).

Discussion

This study is the first to report on the highly significant association between the change in normalized choline during RT and patient survival. Patients with greater than 40% decrease in normalized choline from week 4 of RT to 2 months post-RT have a worse OS and PFS than patients without such a change. Moreover, while previous studies have examined the change in MRS spectra after RT (13), this is one of the first studies to examine the evolution of tumor metabolites during RT.

Although maximal resection followed by concurrent chemoradiotherapy continues to be the standard of care for GBM (2) and most other HGG, this universal approach does not take into account the heterogeneity of these diseases. Traditional prognostic factors such as age, histology, performance status, and extent of resection (14) are not specific enough to allow individualization of treatment. A metabolic marker for treatment response such as MRS could allow further tailoring of therapy, especially since decreases in tumor size or contrast enhancement are not always accurate indicators of response (3, 5, 7). Targeted therapies such as antibodies to vascular endothelial growth factor antibodies are one such treatment which may be used for patients who do not show early evidence of treatment response (15, 16).

Characteristic MRS metabolic profiles suggestive of tumor have been previously described (10, 17, 18) and our findings are consistent with these. In addition, the differences between tumor and normal brain choline/NAA, choline/creatine, and NAA/creatine ratios decreased with time. Whether this represents tumor kill with a return of brain tissue to a pre-tumor metabolic state, or an inability to differentiate tumor from post-irradiated normal brain is unclear. This distinction is important, given that MRS changes can be seen in normal brain even after doses of <6 Gy (19). This has clinical implications for patient follow-up if the ability to differentiate between tumor and post-irradiated normal brain diminish with time.

Serial MRS studies of gliomas have been conducted after treatment with external beam RT (13, 20-22), gamma knife radiosurgery (9), brachytherapy (23), and chemotherapy (20, 24). Our results are consistent with these studies which show that after treatment, there is a characteristic decrease in choline (13, 21-24) with tumor response and an increase in choline with tumor recurrence (9, 13, 25). These changes in choline are thought to reflect tumor burden, since choline is a marker of cell density and cell membrane turnover (11, 26, 27). As expected, we observed a significant increase in normalized choline with time. However, what was more striking was that when patients were separated based on percentage change in normalized choline, there was a highly significant association with OS and PFS. Patients with a greater than 40% decrease in normalized choline between week 4 of RT and 2 months post-RT had worse OS and PFS.

The radiobiologic events which account for this finding may be complex. Choline reflects turnover of the cell membrane during cell proliferation (10, 11). A greater decrease in normalized choline may be related to less tumor proliferation, and thus better prognosis. However, choline may also be a reflection of cell membrane breakdown during cell death. The choline signal detected by MRS is strongly correlated with free choline and phosphocholine, while phosphatidylcholine in intact cell membranes is not (11). Thus, precursors and degradation products of phosphatidylcholine, but not phosphatidylcholine in intact membranes, constitute the choline peak. Therefore, we hypothesize that the choline signal may reflect both cell proliferation as well as cell death with release of free choline moieties. In this model, the decrease in choline through slowed tumor proliferation and reduced cell density may be partially offset by the release of free choline during radiation-induced cell kill. In this manner, a smaller decrease in normalized choline may actually reflect more tumor cell kill by radiation, which could account for the improved outcomes seen in this group. Conversely, a larger decrease in normalized choline, while still reflecting slowed tumor replication and

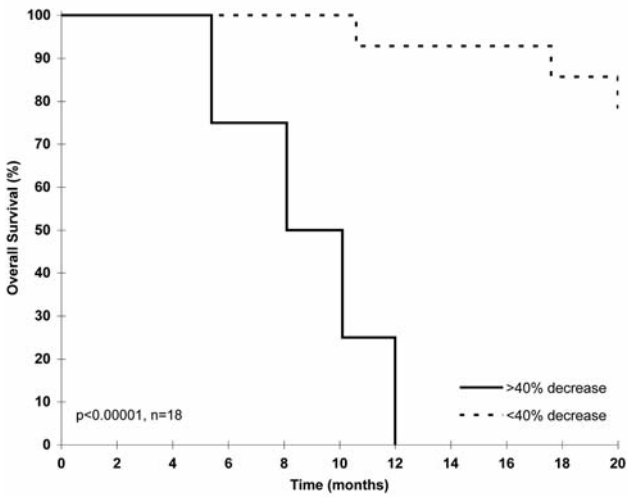

Figure 3. *Kaplan-Meier estimates of overall survival according to percentage change in normalized choline between MRS scans at week 4 during radiotherapy and 2 weeks post-radiotherapy.*

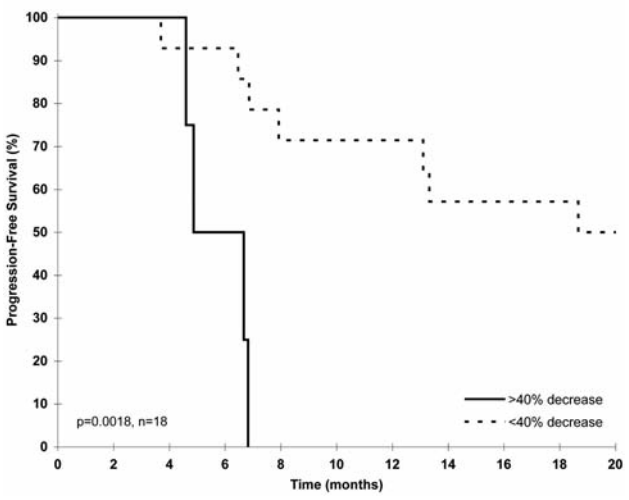

Figure 4. *Kaplan-Meier estimates of progression-free survival according to percentage change in normalized choline between MRS scans at week 4 during radiotherapy and 2 weeks post-radiotherapy.*

reduced tumor cell density, would not be countered by an increased choline signal from cell membrane breakdown, and thus may represent less tumor cell death by radiation.

Another important finding is that there were no significant differences in metabolite ratios between baseline and week 4 of RT. The observed MRS changes took longer than 4 weeks to occur. Thus, MRS changes using these metabolites may not occur early enough to act as an indicator of treatment response within the first 4 weeks of RT. Changes were seen between scans done 2 months post-RT and those done either at baseline or at 4 weeks into treatment.

This study has potential limitations. The study population of patients with HGG, while mostly consisting of glioblastoma patients (17/26), is heterogeneous. However, there is little data to suggest that changes in MRS spectra with RT are significantly different between the various histologies of HGG. Even low-grade gliomas, similarly to HGG, demonstrate the characteristic decrease in choline after treatment (24, 28). Moreover, sensitivity analysis with only patients with GBM confirmed the significance of >40% decrease in choline as a prognostic factor for OS. A second potential issue is post-surgical MRS changes. Because the baseline MRS was performed after surgery, postoperative changes could have altered MRS patterns. However, Tarnawski *et al*. did not observe any correlation between time after surgery and results of MRS, and concluded that MRS after surgery reflects the metabolism of residual tumor or surgical margins, and not post-operative changes (29). Although the number of patients with GTR was small, the poor prognostic effect of a >40% decrease in normalized choline appears to be independent of GTR status. The extent of resection remains controversial in neuro-oncological literature and is confounded by other factors, including performance status and tumor location (30, 31). Finally, this study was underpowered to determine the significance of a >40% decrease in normalized choline independent of the administration of concurrent chemotherapy which became standard of care during the course of this trial (2). This requires further investigation in prospective studies.

The results from this study show promise that MRS can be used to evaluate treatment response at the metabolic level in patients with HGG. Although MRS requires specialized software and expertise, it remains a valuable tool and warrants additional study to identify its role in the diagnosis and treatment of these types of cancer.

In conclusion, the change in normalized choline detected by MRS between week 4 of RT and 2 months post-RT is prognostically important and highly correlated with both OS and PFS. Treatment effects also did not manifest until after the RT was completed. Current techniques may preclude using MRS as an early indicator of treatment response during RT. Additional investigations are needed to verify these findings and clarify the optimal use of the kinetics of choline-containing compounds in the management of patients with HGG.

Conflict of Interest

None.

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