Increased choline signal coinciding with malignant degeneration of cerebral gliomas: a serial proton magnetic resonance spectroscopy imaging study

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The authors tested the hypothesis that proton magnetic resonance spectroscopy (1H-MRS) imaging can be used as a supportive diagnostic tool to differentiate clinically stable brain tumors from those progressing as a result of low- to high-grade malignant transformation or posttherapeutic recurrence. Twenty-seven patients with cerebral gliomas verified on histological examination were studied repeatedly with 1H-MRS imaging over a period of 3.5 years. At the time of each 1H-MRS imaging study, clinical examination, MR imaging, positron emission tomography with 18F-fluorodeoxyglucose, and biopsy findings (when available) were used to categorize each patient as having either stable or progressive disease. Measures of the percentage changes in the choline (Cho) 1H-MRS imaging signal intensity between studies, which were obtained without knowledge of the clinical categorization, allowed the investigators to segregate the groups with a high degree of statistical significance. All progressive cases showed a Cho signal increase between studies of more than 45%, whereas all stable cases showed an elevation of less than 35%, no change, or even a decreased signal. The authors conclude that increased Cho levels coincide with malignant degeneration of cerebral gliomas and therefore may possibly be used as a supportive indicator of progression of these neoplasms.

Key words • primary brain tumor • cerebral glioma • malignant degeneration • tumor recurrence • magnetic resonance spectroscopy • proton magnetic resonance spectroscopy imaging • choline

The clinical challenge of managing primary brain tumors is still quite formidable. The need for prompt and accurate recognition of a low- to high-grade progression and/or of a posttherapeutic recurrence in cerebral gliomas has given impetus to continued reassessment and to the search for novel neuroimaging methods. Although computerized tomography (CT) and magnetic resonance (MR) imaging can solve many diagnostic problems related to brain tumors, they do not provide the biological information critical to the appropriate management of cerebral gliomas. Positron emission tomography with 18F-fluorodeoxyglucose (FDG-PET) provides insights into tumor cell metabolism and has emerged as a powerful method to assess the biological behavior of cerebral gliomas. However, the fact that PET facilities are available in only a few specialized centers has severely limited the widespread adoption of the FDG-PET method to manage cerebral gliomas. The wider availability of single photon emission CT (SPECT) scanners has intensified the search for SPECT tracers suitable to evaluate gliomas. Reviews of the proposed radionuclide alternatives (PET and SPECT) are available in the literature.

In contrast to the scarcity of PET equipment and its ancillary services, high-field (1.5- and 2-tesla) MR imaging devices are found in most medical centers and enable proton magnetic resonance spectroscopy (1H-MRS) and its variant 1H-MRS imaging to be performed. Recently, a large number of reports describing both 1H-MRS and 1H-MRS imaging have indicated that this technique offers biological information about brain tumors. Of particular interest is the report by Preul, et al., who presented data indicating that 1H-MRS imaging can assist in determining the histopathological type of brain tumor. Other studies contain preliminary data on the application of 1H-MRS imaging to monitor therapy for such neoplasms. It is apparent that 1H-MRS imaging may offer supportive evidence to identify deterioration caused by recurrence or malignant degeneration.

In this study we tested the hypothesis that serial 1H-MRS imaging can help detect malignant degeneration and/or the recurrence of brain gliomas. We refer to these
two related phenomena as “progression.” This hypothesis is based on our previous observations of individual cases, which indicate that the choline (Cho) signal increases with tumor progression. At the time of the repeated 1H-MRS imaging examinations, patients were categorized with either stable or progressive disease, based on the preponderance of information derived from the neurological examination, MR imaging, FDG-PET scanning, and histological studies (in the majority of the patients with progressive disease). Our aim was to evaluate if the change in Cho signal intensity relative to the most recent 1H-MRS imaging study could segregate progressive from stable cases.

Clinical Material and Methods

Neuroimaging Studies

We used multisection 1H-MRS imaging, which allows simultaneous acquisition of spectral signal intensities from four 15-mm sections divided into a number of 0.84-ml single-volume elements. The acquired data can be displayed in a tomographic format, thus making 1H-MRS imaging particularly suitable to explore the metabolic heterogeneity of intracranial tumors. The 1H-MRS imaging method allows us to recognize several endogenous brain chemicals. The principal metabolite signals in the long echo time 1H-MRS imaging are from N-acetyl-containing compounds (N-acetyl aspartate [NAA] as the prominent contributor); Cho-containing compounds; creatine plus phosphocreatine (CR); and lactate (LAC). Among the chemicals detected by 1H-MRS imaging, Cho is likely to be the most reliable indicator of malignancy in human gliomas. The elevated Cho signal probably reflects an increase in metabolites that are precursors of the membrane phospholipids needed to support neoplastic proliferation, as well as compounds involved in the rapid cellular turnover.

The multisection (four-slice) 1H-MRS imaging studies were performed with a 1.5-tesla MR imager equipped with self-shielded gradients using an established data acquisition procedure. Phase encoding was used to obtain a 32 × 32-element array of spectra from voxels having a nominal volume of 0.84 ml (7.5 × 7.5 × 15 mm) within the selected slices. The 1H-MRS imaging data acquisition comprised a multiple-slice spin-echo slice selection with a repetition time of 2300 msec and an echo time of 272 msec. Outer-volume signal saturation was used to suppress signals arising from the skull marrow and surface tissues. Four 15-mm-thick slices with 3-mm inter-slice spacing were acquired. After completing the 1H-MRS imaging, a series of T1-weighted images were obtained with the same field of view and oblique angulation used for the 1H-MRS image acquisition. Contiguous slicing (3-mm thickness) was used so that a total of five 3-mm images from the MR series corresponded to any single 15-mm 1H-MRS imaging slice.

The 1H-MRS imaging reconstruction was performed on a SPARC-II workstation (Sun Microsystems Computer Corp., Mountain View, CA) using specially designed software. The data sets, which were reconstructed one slice at a time, were zero-filled to 32 × 32 spatial and 512 time domain elements. The spatial dimensions were filtered with a sine function and the time dimension was filtered with a 2-Hz exponential filter. Fourier transformation in all three dimensions resulted in a 32 × 32-element array of spectra. To facilitate rapid automatic processing, further calculations were performed with magnitude-corrected spectral data. Signal intensity images were produced by determining the signal within 0.2 ± 0.1 ppm of the expected location of the NAA, Cho, CR, and LAC signals. Spectroscopic voxels showing poor spectral resolution (less than half-height separation of Cho and CR signals) or residual water and/or lipid signals were excluded.

Tumor regions of interest (ROIs) containing at least two spectroscopic voxels (7.5 × 7.5 × 15 mm) were selected from each study to include the area of highest Cho signal within the region exhibiting elevated T2 weighting. In those cases in which no such area could be identified on the 1H-MRS images, the tumor ROIs were selected from the T2-weighted images. To calibrate the signal intensities from different imaging studies and individuals to a common scale, the signal amplitude of each metabolite (NAA, Cho, CR, and LAC) in the tumor ROI was normalized to the corresponding amplitude in matching ROIs from a normal area of the contralateral brain. Metabolite signal intensity ratios (NAA/Cho, NAA/CR, Cho/CR) were also calculated for each ROI.

A routine MR contrast study was performed in each patient separately within 2 days (before or after) of the 1H-MRS imaging examination. The FDG-PET images were obtained according to well-established procedures.

Study Protocol

All patients were studied by using a protocol approved by the National Institutes of Health and informed consent was obtained from each participant before inclusion in the study. Twenty-seven patients who were referred for neuroimaging evaluation of verified cerebral gliomas participated. Over a period of 3.5 years, the 27 patients were tested 72 times. The clinicopathological features of the tumors, intervals from previous treatment to the time of the imaging studies, and the results of these studies (MR, FDG-PET, and 1H-MRS imaging) are reported in Tables 1 (patients with stable disease) and 2 (patients with progressive disease). The clinical, MR imaging, and FDG-PET findings are presented as differences from the previous study. The 1H-MRS imaging findings are presented both as individual study values and as between-studies percentage changes in normalized Cho.

In all patients the initial diagnosis was formulated from a histopathological examination of tissue specimens obtained from biopsy procedures. In most cases these evaluations were performed at other institutions prior to referral. An experienced neurologist (R.R.), who was unaware of the 1H-MRS imaging results, categorized the cases as being stable (16 patients) or progressive (11 patients) at the time of each 1H-MRS imaging examination. All patients classified with stable disease presented with unchanged clinical status since the previous examination. The patients with progressive disease showed either malignant degeneration of an untreated low-grade lesion or recurrence of a previously treated tumor. The categorization of the patients was based on the 45 repeated studies, and the first examination was used as the baseline. All...
available data at the time of the repeated examination, such as neurological examination, MR imaging, FDG-PET, and biopsy results were used to categorize the patients. In the progressive group, a malignant transformation was proven on biopsy studies in seven of the 11 cases.

The time from onset of disease to imaging was $48.5 \pm 6 40.9$ months (mean $\pm$ standard deviation [SD]) in the whole study population, $54.8 \pm 46.7$ months in the stable, and $36.1 \pm 21.4$ months in the progressive group. Cases in which the referring clinician suspected disease progression tended to be referred far more frequently, whereas cases that were clinically stable over time were referred less frequently. The between-studies interval was $8.3 \pm 5.1$ months in the whole study population, $14 \pm 5.6$ months in the stable group, and $7.8 \pm 4$ months in the progressive group. Statistical analysis was performed using the two-tailed t-test and percentile rankings. No verified case of radiation necrosis was observed during the period of this study.

### TABLE 1

<table>
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<tr>
<th>Case No.</th>
<th>Age (yrs), Sex</th>
<th>Diagnosis</th>
<th>Mos to Disease Onset</th>
<th>Mos to Treatment</th>
<th>Clinical Data</th>
<th>MR Imaging</th>
<th>FDG-PET</th>
<th>Cho on 1H-MRS Imaging</th>
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* Astro = astrocytoma; C = chemotherapy; E = enhancing; hyper = hyperintense; hypo = hypointense; I = improved; N = nonenhancing; oligo = oligodendroglioma; RT = radiotherapy; S = stable; Sx = surgery.

† No comparison with radiological studies was performed at baseline.
Results

Figures 1 and 2 show consecutive MR and 1H-MRS images (including NAA, Cho, CR, and LAC) obtained in two representative patients. Figure 1 depicts consecutive MR and 1H-MRS images obtained in a patient with stable disease (Case 7). In both studies, the NAA images demonstrate a signal void relative to the contralateral hemisphere that encompasses most of the region of abnormal signal intensity on the T2-weighted image. The Cho images show a signal enhancement relative to the contralateral hemisphere that is located in the center of the lesion depicted with T2 weighting. The size of the area of Cho enhancement appears smaller in the second study, likely because of the slight differences in slice location or angulation. The CR image in the repeated study shows a minor area of enhancement relative to the contralateral hemisphere that was not evident in the baseline study. Also, the LAC images in both studies show peripheral areas of artifactual signal elevation caused by incomplete suppression of lipid signals arising from bone marrow and extracranial tissues. This resulted from signal that could not be assigned to LAC by using established criteria (chemical shift should show a doublet centered at 1.33 ppm) after visual inspection of spectra from these voxels (data not shown).

Figure 2 shows consecutive MR and 1H-MRS images obtained in a patient with progressive disease (Case 22). We selected this patient specifically to illustrate the complexity and heterogeneity that can be found in 1H-MRS imaging in a case of recurrent brain tumor with the lowest change (46%) in normalized Cho between two consecutive studies. The repeated 1H-MRS imaging study (Fig. 2B) demonstrates that disease progression was characterized by an increased Cho signal, as well as by an increased CR signal, a decreased NAA signal, and the presence of LAC. The NAA image shows a large area of signal void relative to the contralateral hemisphere, corresponding to most of the region of abnormal signal intensity on the T2-weighted image. The Cho image exhibits signal enhancement in a limited area of the lesion depicted with T2 weighting. The CR image shows a signal enhancement that coincides with the area of Cho enhancement. The LAC image shows the presence of LAC in the mesial regions of the lesion depicted with T2 weighting. As mentioned previously, the LAC images demonstrate an artifactual signal elevation at the brain periphery in both studies. We have presented only the normalized Cho findings because the normalized NAA, CR, and LAC, as well as the within-voxel metabolite ratios (NAA/Cho, NAA/CR, Cho/CR) showed no association with the patient’s categorization.

Figure 3 illustrates the percentage change in normalized Cho intensity that was observed between two consecutive studies for the two groups. In the stable group, the between-studies percentage changes of the normalized Cho were always below 35 (range −33 to +28%, mean ± SD, 0.4 ± 13.2%). In several instances, the percent age changes in normalized Cho took a negative direction. Because of the heterogeneity of previous treatments and the
differences in time from treatment in our study group, we could not discriminate possible therapy-related improve-
ments of the disease from intraindividual variability of the 
1H-MRS imaging. In the group with progressive disease, 
the between-studies percentage changes in the normalized 
Cho were always above 45 (range 46–104%, mean ± SD, 
55.6 ± 33.2%). The diagram aptly demonstrates how well 
the percentage change in normalized Cho intensity segre-
gates the two groups: a critical value of 35% completely 
segregates them. A t-test showed a statistically significant 
(p < 0.00005) difference between the stable and progres-
sive groups.

Patients with glioma who develop malignant degenera-
tion or malignant recurrence are known to have a statisti-
cally poor prognosis for survival, whereas patients with 
stable disease (an initial low-grade diagnosis or effective 
treatment) have longer survival times. Hence, a survival 
review may indicate whether the patients were accurately 
categorized. A survival review performed at the end of the 
study showed that all but one patient (Case 11) in the 
group with stable disease had survived, whereas all but 
one patient (Case 27) in the group with progressive dis-
ease had died. This finding is consistent with expectations, 
indicating that the clinical categorization at the time of 
imaging studies was accurate.

Discussion

Morphological studies using CT and MR imaging, and 
physiological imaging using PET and SPECT scanning 
have played a pivotal role in defining landmarks used 
to manage primary brain tumors clinically. However, as 
indicated earlier, many questions regarding the care of 
patients with cerebral gliomas remain unanswered. The 
addition of complementary biochemical information, as 
provided by 1H-MRS imaging, could lead to further ad-
vances in patient management. Repeated 1H-MRS im-
ing examinations provide a noninvasive method to de-
termine whether a patient is affected by a progressive 
neoplasm that should be treated or is in stable condition. 
Undoubtedly, the widespread availability of high-field 
(1.5- and 2-tesla) MR imagers will help make this proce-
dure available to and accepted by those physicians who 
manage cases of primary brain tumors.

In this study, MR imaging and FDG-PET together with 
the neurological examination and the biopsy findings
in seven of 11 progressive cases) were used to establish whether a particular patient was exhibiting a malignant degeneration. Our results indicate that serial 1H-MRS imaging effectively and accurately differentiates between stable and progressive disease. The 1H-MRS imaging data not only show a difference in distribution (means) between the two groups, but also a high discriminatory power, in that any threshold between 35% and 45% of between-study percentage change in normalized Cho yields separation between stable and progressive cases.

The 1H-MRS imaging method has two major limitations that account for the high rate (approximately 30%) of technically inadequate studies. First, the metabolite signals from some regions (for example, posterior fossa, medial temporal lobes, and superior medial gyri) are broadened by partial magnetic field inhomogeneities caused by the proximity of sinuses and bones. Second, the signal/noise ratio can be affected by head motion during the acquisition phase.

Establishing which compounds contribute to the Cho signal has been an area of active research in recent years. If the major water-soluble Cho-containing compounds in the brain are added together, their total concentration does not account for the large signal that is seen in vivo. This has led to the conclusion that relatively immobile lipid molecules, such as phosphatidylethanolamine, can be seen in vivo. Conversely, in a 1994 in vivo study of the canine brain, the Cho signal was attributed predominantly to water-soluble glycerophosphocholine and phosphocholine. A recent report on 18 patients with neoplastic and infectious brain lesions stated that the in vivo Cho signal correlated with in vitro measures of cellular density and water-soluble Cho-containing compounds (free Cho, phosphocholine and glycerophosphocholine), but not with membrane-bound phosphatidylcholine. All these Cho-containing compounds participate in phospholipid metabolism. Thus, the increased Cho peak found in most of the 1H-MRS and 1H-MRS imaging studies of brain tumors has been attributed to a greater membrane synthesis, increased cellularity, or to a rapid cell turnover. Our observations are consistent with these views. An increase in Cho paralleling clinical deterioration is consistent with increased cellularity, as well as with increased cell turnover and phospholipid metabolites.

In this study, the criterion for ROI selection was unique. We hypothesized that the “hottest” Cho region represents the site of highest tumor malignancy. Until now, investigators have used average readings over the entire tumor volume. The “worst” voxel analysis method we used is analogous to the pathological examination, in which the diagnosis is derived from the worst area of the biopsy specimen. This seemed logical despite the large difference between histopathological and 1H-MRS imaging resolution. The promising results of this study support the valid-

**Fig. 2.** Magnetic resonance and 1H-MRS images obtained on the first (upper) and second (lower) examination in a patient with progressive disease showing the lowest between-studies change in normalized Cho. For color scales, see Fig. 1. For a detailed discussion of the imaging example see the Results section.
of incomplete volumetric localization. In the present study, LAC was occasionally found in some high-grade tumors (Fig. 2), but its presence and changes between examinations did not help us differentiate between the two groups of patients.

In the present analysis the metabolite signal intensities were measured in the tumor and in the healthy contralateral brain to form between-voxel ratios. In addition, within-voxel metabolite signal intensity ratios (NAA/Cho, NAA/CR, Cho/CR) for each ROI were determined. We believe that the absence of significant results when the within-voxel ratio is used reflects the limitations of estimating metabolite changes by ratios when a simultaneous change of two or more metabolites occurs. This could also be related to the limited value of NAA and CR to differentiate between the two groups of patients. The use of between-voxel ratios, which proved to be the most informative, is validated by our previous work, in which we demonstrated that in healthy adults there are no statistically significant side-to-side differences for any of the reported metabolites.52,53

With this study we could not answer the very relevant question of the differential diagnosis between radiation necrosis and tumor recurrence, simply because we lacked cases with verified, pure radiation necrosis. We encountered one patient (Case 23) in whom a biopsy showed the coexistence of tumor recurrence and radiation necrosis. The differential diagnosis could be formulated only at the microscopic level, which is beyond the resolution of H-MRS imaging.

Conclusions

This study shows that serial H-MRS imaging can be used to detect glioma progression. We anticipate that this noninvasive method may play a role in improving the management of patients with cerebral gliomas.

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References


Fig. 3. Scatterplot showing percentage change in normalized Cho signal intensity that was seen between two consecutive studies for the two groups of patients.


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