Serial analysis of 3D H-1 MRSI for patients with newly diagnosed GBM treated with combination therapy that includes bevacizumab

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Abstract Interpretation of changes in the T1- and T2-weighted MR images from patients with newly diagnosed glioblastoma (GBM) treated with standard of care in conjunction with anti-angiogenic agents is complicated by pseudoprogression and pseudoresponse. The hypothesis being tested in this study was that 3D H-1 magnetic resonance spectroscopic imaging (MRSI) provides estimates of levels of choline, creatine, N-acetylaspartate (NAA), lactate and lipid that change in response to treatment and that metrics describing these characteristics are associated with survival. Thirty-one patients with newly diagnosed GBM and being treated with radiation therapy (RT), temozolomide, erlotinib and bevacizumab were recruited to receive serial MR scans that included 3-D lactate edited MRSI at baseline, mid-RT, post-RT and at specific follow-up time points. The data were processed to provide estimates of metrics representing changes in metabolite levels relative to normal appearing brain. Cox proportional hazards analysis was applied to examine the relationship of these parameters with progression free survival (PFS) and overall survival (OS). There were significant reductions in parameters that describe relative levels of choline to NAA and creatine, indicating that the treatment caused a decrease in tumor cellularity. Changes in the levels of lactate and lipid relative to the NAA from contralateral brain were consistent with vascular normalization. Metabolic parameters from the first serial follow-up scan were associated with PFS and OS, when accounting for age and extent of resection. Integrating metabolic parameters into the assessment of patients with newly diagnosed GBM receiving therapies that include anti-angiogenic agents may be helpful for tracking changes in tumor burden, resolving ambiguities in anatomic images caused by non-specific treatment effects and for predicting outcome.

Keywords Metabolic imaging · MRSI · Newly diagnosed glioblastoma · Anti-angiogenic therapy · Survival

Introduction

The current standard of care for patients with newly diagnosed glioblastoma (GBM) is maximal safe resection, followed by radiation therapy and temozolomide [1]. A variety of investigational agents are being considered in conjunction with this strategy in order to improve outcome. Evaluation of combination therapies is complicated by differences in their mechanisms of action that lead to difficulties in assessing the spatial extent of tumor and ambiguities in the interpretation of conventional anatomic images. One such effect that has been reported to occur in up to 30% of patients treated with radiation and temozolomide is pseudoprogression, which is characterized by an increase in the size of
the enhancing lesion that subsequently disappears without further intervention [2].

Given that GBM are characterized by abnormal vasculature, there has been great interest in using treatment regimens that include anti-angiogenic agents [3–5]. The dramatic reduction in the size of the contrast enhancing (CE) lesion that was observed following treatment of recurrent GBM with bevacizumab was very encouraging and led to conditional approval for its use. Subsequent studies in populations of patients with newly diagnosed GBM have shown longer progression free survival but have not established a significant improvement in overall survival [6, 7]. For cases where the treatment regime includes such anti-angiogenic agents, it is not clear whether early reductions in the CE lesion are indicative of successful treatment or if they are associated with pseudo- or true response.

The ambiguities in interpreting changes in the size of the CE lesion have highlighted the need to develop alternative approaches for assessing treatment response. The RANO criteria are a first step in that direction [8], as they consider changes in the cross sectional diameter of both the CE and T2 lesion. A further suggestion has been that estimating the three dimensional (3D) volumes of the anatomic would provide a more precise assessment of their absolute and relative changes with time. While providing more precise quantification of lesion size is definitely of interest, it is not clear whether this would be able to address pseudoprogression and pseudoresponse. Having an imaging modality that makes it possible to track biological response to therapy is likely to be a more promising approach. Previous studies that applied H-1 MR spectroscopic imaging (MRSI) to evaluate patients with GBM have shown that the magnitude and spatial extent of regions with abnormal metabolism is often quite different from areas corresponding to the CE and T2 lesions [9–12]. This suggests that metabolic imaging can provide complementary information about changes in tumor burden that would be helpful in assessing response to therapy.

The purpose of this study was to obtain 3D H-1 MRSI data from a cohort of patients with newly diagnosed GBM being treated with radiation therapy, temozolomide, erlotinib and bevacizumab in order to evaluate treatment induced changes in the metabolic characteristics of the lesion. The hypothesis being tested was that levels of choline, creatine, N-acetylaspartate (NAA), lactate and lipid change in response to treatment and that metrics describing these characteristics are associated with survival. External beam radiation therapy (RT) was initiated within 5 weeks of diagnosis and delivered an average dose of 60 Gy to the tumor site in 2-Gy fractions over a 6-week period. Temozolomide was given concurrently and after RT at a daily dose of 75 mg/m². Patients who were not on anti-epileptic drugs received 150 mg/day of erlotinib and patients who were on anti-epileptic drugs received 500 mg/day starting on day 1 of RT. Bevacizumab was prescribed at a dose of 10 mg/kg IV every 14 days starting in week 2 of RT. All patients participating in the study gave informed consent, according to the guidelines of our institutional review board. Overall survival (OS) was calculated as the time to death from the start of treatment and progression free survival (PFS) as the time from the start of treatment to the examination at which the patient was determined to have progressed based upon the RANO criteria or the time of death if there had been no prior progression [8].

Imaging protocol

Patients received MR exams at multiple time points, including following surgical resection but prior to RT (pre-RT), between 3 and 5 weeks into treatment (mid-RT), within 2 weeks after completion of RT (post-RT), and at the next follow-up scan that was approximately 4 months after the baseline time point (Fup1). All scans were obtained with a 3T MR scanner (GE Healthcare, Milwaukee, WI), the body coil for transmission and an 8-channel phased array coil for reception. Standard anatomical imaging comprised axial T2-weighted fluid attenuated inversion recovery (FLAIR) images (TR/TE/TI = 9500/122/2375 ms, matrix = 256×256, slice thickness = 3 mm, FOV = 24×24 cm), and pre- and post-contrast volumetric T1-weighted inversion recovery spoiled gradient echo images (TR/TE = 8.86/2.50 ms, matrix = 256×256, slice thickness = 1.5 mm, FOV = 24×24 cm, TI = 400 ms, Flip angle = 15°).

Lactate edited 3D H-1 MRSI data [14] were obtained with CHESS water suppression, very selective saturation (VSS) outer volume suppression and PRESS volume selection (TR = 1104 or 1300 ms, TE = 144 ms, acquisition matrix of 16×16×16 or 18×18×16, nominal spatial resolution of 1 cm³). Phase encoding was applied in the right-left and anterior-posterior directions with fly-back trajectories in the superior-inferior direction. An over-PRESS factor of 1.5 was applied with VSS bands to avoid chemical shift artifacts and to suppress residual lipid signals. The total acquisition time was 9.5 min, with 712 complex spectral points read indirectly using the EPI train of gradient pulses and spectral bandwidth of 988 Hz. The PRESS selected volume was defined using the anatomic images and generally covered the lesion and 200–500 cc of normal tissue. Areas with sharply varying magnetic susceptibility and lipid contamination were avoided whenever possible.

Methods

Thirty-one patients with pathologically confirmed newly diagnosed GBM, who were being treated as part of a single institution Phase II clinical trial [13] were recruited for this study.
developed to describe the deviation of choline and NAA in regions with normal metabolite levels in the same individual [19, 20]. The calculation is performed using an automated, iterative procedure that uses choline and NAA levels in the spectral array from each examination separately and is independent of the anatomic images. Once the CNI map had been generated for each patient and time point, their metabolic lesion was defined as the region having values greater than or equal to 2 (CNI2). Figure 1 shows an example of the spectral data and CNI overlays from the Fup1 scan for a patient with a relatively large metabolic lesion at this time point. The CNI intensities were interpolated to the resolution of the anatomic images for display purposes and the overlay was thresholded to show only values above 2 in order to highlight the spatial extent of the metabolic lesion. The choline to creatine index (CCrI) was estimated in a similar manner, but with the definition of abnormal voxels being based upon those that had been identified during the CNI calculation.

To be able to compare across serial exams, levels of choline, creatine, and NAA intensities were normalized by their median value in voxels from normal appearing brain (nCho, nCr, and nNAA). The spectral data were processed as described previously [17, 18] to provide levels of choline, creatine, NAA, lactate and lipid. The choline-NAA index (CNI) is a metric processed to describe the deviation of choline and NAA in regions with normal metabolite levels in the same individual [19, 20]. The calculation is performed using an automated, iterative procedure that uses choline and NAA levels in the spectral array from each examination separately and is independent of the anatomic images. Once the CNI map had been generated for each patient and time point, their metabolic lesion was defined as the region having values greater than or equal to 2 (CNI2). Figure 1 shows an example of the spectral data and CNI overlays from the Fup1 scan for a patient with a relatively large metabolic lesion at this time point. The CNI intensities were interpolated to the resolution of the anatomic images for display purposes and the overlay was thresholded to show only values above 2 in order to highlight the spatial extent of the metabolic lesion. The choline to creatine index (CCrI) was estimated in a similar manner, but with the definition of abnormal voxels being based upon those that had been identified during the CNI calculation.

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Fig. 1 Anatomic images, spectra and CNI color overlays from the Fup1 scan for a 47 year old female patient with a KPS of 90, sub-total resection, a low PFS of 159 days and a low OS of 206 days. The CNI maps were thresholded to show only values that were 2 or higher in order to highlight the metabolic lesion. The median and maximum CNI were 4.8 and 19.9. Note the high lipid peaks in the cavity, which are surrounded by areas with increased choline and decreased NAA that corresponded to the metabolic lesion. The residual CE volume was 3 cm³ is minimal and corresponds mainly to a thin rim around the resection. The volume of the T2 lesion at this time point is 29 cm³. As is shown from the FLAIR images and CNI overlays from the three lower slices, the metabolic lesion is relatively large and gives a clear picture of regions that are likely to reflect recurrent/residual tumor.
nCr and nNAA). Signals from lipid and lactate peaks were normalized with the median NAA from voxels in normal appearing brain (nLac and nLip). Parameters considered for analysis were the volume of the CNI2 and the median, maximum and summation of voxel values for the CNI, CCrI, nLac and nLip from within the CNI2 regions at each of the four time points.

**Statistical analysis**

Serial changes in imaging parameters were evaluated using Wilcoxon signed rank sign tests for paired analysis. Association with PFS and OS was assessed using Cox proportional hazards analysis, taking into account patient age and extent of resection. The cut-off for defining a significant result in this exploratory study was a P value of 0.05.

**Results**

The median PFS for these patients was 404 days with 27 events and 4 subjects censored. The median OS was 603 days with 23 events and 8 subjects censored. There were 16 females and 15 males: median age was 52 years with a range of 21 to 75, median KPS was 90 with a range of 60–90. There were 9 patients who were designated based upon clinical criteria as having received a gross total resection, 17 who had a sub-total resection and 5 who were characterized as having a biopsy.

**Changes in anatomic lesion volumes**

For the 22 patients who had anatomic scans at all 4 time points there were overall reductions in the volumes of the anatomic lesions with treatment. The mean pre-RT T2 lesion volume was 36.2 cm³, which subsequently decreased to 28.9 cm³ at mid-RT (p > 0.05), to 15.3 cm³ at post-RT (p < 0.0001) and to 14.7 cm³ at Fup1 (p = 0.0002). The mean CE lesion volume decreased from 5.2 cm³ at pre-RT to 3.0 cm³ at mid-RT (p = 0.018), 2.1 cm³ at post-RT (p = 0.0002) and 0.7 cm³ at Fup1 (p < 0.0001). The mean T1s volume was obtained by thresholding the T1 subtraction image and showed similar changes to the CE lesion volumes but was overall larger in size at all four time points (mean volumes of 9.4, 5.8, 3.3 and 1.7 cm³ respectively). These results are represented in the bar graphs in Fig. 2.

**Changes in metabolic parameters**

At pre-RT, the CNI2 lesion volumes were much larger than the CE and T1s lesions (mean of 25.2 vs. 5.2 and 9.1 cm³ respectively). Although many of the T2 lesion volumes at pre-RT were larger than the CNI2 lesions, the mean overlap was only 11.2 cm³. This indicates that there were sub-regions of FLAIR hyperintensity with CNI <2, as well as regions of normal appearing brain with elevated CNI. For the 19 patients with metabolic imaging data at all 4 scans, the changes in mean CNI2 volume from pre-RT to post-RT and from pre-RT to Fup1 were significant (from 25.2 to 16 cm³, p = 0.0017 and 17.2 cm³, p = 0.0145 respectively). The differences in spatial extent of the anatomic and metabolic lesions became even more extreme after treatment, with the overlap between the CNI2 and T2 lesion volumes being 4.6 cm³ at post-RT and 3.8 cm³ at Fup1. As can be seen from the lower bar graphs in Fig. 2, the sum(CNI), sum(CCrI), sum(nLac) and sum(nLip), which represent how abnormal the metabolite levels were within the CNI2 region from each time point relative to values in normal brain showed a similar pattern of changes.

**Serial results from individual patients**

Figure 3 shows an example of the serial anatomic images and overlays of CNI maps from pre-RT, post-RT, Fup1 and at two later time points prior to progression for a 47 year old patient who had received a sub-total resection, and had a KPS of 70, with PFS of 330 days and OS of 706 days. The images on the upper panel are from the post-contrast T1-weighted sequence and show that the enhancing lesion virtually disappears by post-RT (from an overall volume of 6.7 cm³ at pre-RT to 0.5 cm³ at Fup1) and a small region of new enhancement appears in the corpus callosum at the 11 month follow-up scan. The FLAIR images on the bottom panel show a large T2 lesion at pre-RT (overall volume of 55 cm³) with two components, one being brighter and the other being less intense. The T2 lesion was smaller at the post-RT and Fup1 scan but was beginning to impinge upon the corpus callosum at the 8 month scan and then continued expanding in the 11 month scan. The CNI2 region represented by the color overlay images in the middle panels included only a portion of the T2 lesion. The maximum CNI at pre-RT was 20.5 and at Fup1 it was 18.6, suggesting that, despite the lack of enhancement, there was substantial residual tumor. On the 8 month and 11 month scans the CNI2 lesion increased in size and had clearly crossed the corpus callosum, but was still smaller than the overall T2 lesion.

Figure 4 shows a similar time course for a second patient who was 38 years old, had a KPS of 90, a biopsy only, similar PFS of 360 days and a shorter OS of 434 days. The overall CE lesion volume at the pre-RT scan was 5.5 cm³ but reduced to <1 cm³ on subsequent scans. The T2 lesion had an overall volume of 37 cm³ at pre-RT, reducing at the post-RT scan and 4 month scans to 25 cm³ and 20 cm³ respectively. At the 11 month scan, the T2 lesion volume was similar in size on the axial slice shown but had expanded on inferior slices. The metabolic lesion had high CNI values at all time...
for PFS \( (p = 0.029) \). In contrast, a large number of the metabolic imaging parameters considered in the analysis were associated with PFS and OS at the Fup1 (4 month) scan (see Table 1). If the population was divided into cohorts with survival either shorter or longer than the median value, it was observed that there were significant reductions in metabolite levels for both cohorts and that these changes were observed at both the post-RT and the Fup1 scans. This was true when using both the median PFS and median OS to define the cut-points.

**Discussion**

The results of our study highlighted the substantial changes in anatomic lesion volumes that are seen in treatments that include anti-angiogenic agents such as bevacizumab. The RANO criteria for assessing response to therapy in patients
An alternative approach that has been suggested for assessing changes in tumor burden is to use 3-D H-1 MRSI to evaluate regions with abnormal metabolism [22–24]. The CNI and related metrics such as the ratio of choline to NAA have previously been shown to be increased in tumor [20], and to detect areas of infiltrative tumor outside of the CE lesion [21]. This may be important for targeting radiation and other focal therapies, as well as monitoring response to treatment [22–29]. Of particular interest is our prior study of patients with newly diagnosed GBM being treated with radiation and temozolomide [12], which indicated that metabolic parameters reduced from pre- to post-RT and that values obtained from the post-RT scan were associated with overall survival.

The results of the current study indicate that there is only partial overlap between the CNI2 and T2 lesions, with the volumes of the CE and T1 subtraction lesions being significantly reduced at the mid-RT scan and continuing to decrease for the next two scans. These observations are consistent with prior reports of changes in lesion volumes that are observed following treatment with anti-angiogenic agents and have been attributed to normalization of the vasculature in the tumor and associated reduction in edema [2–7]. Of interest is that the only anatomic parameter associated with PFS was the volume of the enhancing lesion at the post-RT scan. It is presumably a reflection of whether the lesion was sensitive and showed an immediate response to this agent.

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Fig. 4 Serial post-Gad T1-weighted, FLAIR images and overlaid CNI maps for a female patient who was 38 years old, had a PFS of 360 days and OS of 434 days. The yellow boxes indicating PRESS selected volumes are oblique for follow-up scans because the 3D imaging and spectral data were post-processed to register them to the pre-RT exam in order to aid in making visual comparisons.

Table 1 Association of imaging and metabolic parameters with survival based upon Cox proportional hazards analysis, adjusting for age and extent of resection.

| Parameters       | Progression free survival | Overall survival |
|------------------|----------------------------|------------------|
|                  | Pre-RT | Mid-RT | Post-RT | Fup1 | Pre-RT | Mid-RT | Post-RT | Fup1 |
| Anatomic imaging |         |        |        |      |         |        |        |      |
| Volume (T2)      | NS      | NS     | NS     | NS   | NS      | NS     | NS     | NS   |
| Volume (CE)      | NS      | NS     | 0.0299 | NS   | NS      | NS     | NS     | NS   |
| Volume (T1s)     | NS      | NS     | NS     | NS   | NS      | NS     | NS     | NS   |
| Metabolic imaging |       |        |        |      |        |        |        |      |
| Volume(CNI2)     | NS      | NS     | NS     | 0.0046 | NS      | NS     | NS     | 0.0080 |
| Sum(CNI)         | NS      | NS     | NS     | 0.0034 | NS      | NS     | NS     | 0.0033 |
| Sum(CrO)         | NS      | NS     | NS     | 0.0033 | NS      | NS     | NS     | 0.0040 |
| Sum(nLac)        | NS      | NS     | NS     | 0.0019 | NS      | NS     | NS     | 0.0010 |
| Sum(nLip)        | NS      | NS     | NS     | 0.0001 | NS      | NS     | NS     | 0.0034 |

Metrics with p<0.05 were considered to be significant but all of the metabolic parameters have much smaller P values. The number of subjects with anatomic imaging data at the each of the time points was 29, 25, 25 and 27, while the number of subjects with metabolic imaging data was 28, 24, 21 and 26. There were 4 subjects censored for PFS and 10 subjects censored for OS.
being regions of FLAIR hyperintensity that are not metab-
olically abnormal and regions of normal appearing brain
with elevated CNI values. This suggests that the spatial
information provided by the metabolic data is complemen-
tary to both types of anatomic images. The color overlays
of the CNI maps that are presented in the figures are helpful in
relating the metabolic and anatomic data in a visual manner.
A critical factor to note here is that although 9/31 patients in
our study were assessed based on clinical criteria as having
a gross total resection, all of the subjects had regions with
metabolic parameters that were consistent with tumor.

Another property of the MRSI data is that they provide
measurements of levels of multiple metabolites that can be
quantified and tracked with time. The reductions in param-
eters reflecting abnormal levels of choline relative to NAA
(CNI) and creatine (CCrI) suggest that the tumor cellularity
is decreasing and is therefore responding to therapy. Prior
studies that used MRSI to study patients being treated with
cediranib [30], radiation and temozolomide [12] and other
treatment strategies [13, 31, 32] have also showed a reduc-
tion in choline related parameters. The lower levels of lipid
and lactate (nLip and nLac) within the metabolic lesion at
the post-RT and the next follow-up scans indicate that the
tumor is becoming better oxygenated, which is consistent
with the impact of vascular normalization [21]. The asso-
ciations of metabolic parameters from the follow-up scan
with PFS and OS are encouraging in terms of providing
surrogate measures of the effectiveness of different treat-
ments. Including consideration of these metabolic param-
eters into the RANO criteria would be especially helpful
for circumstances where the T2 lesion has increased in size
and it is uncertain whether this is due to tumor progression.
While the spatial resolution of MRSI is currently limited
by signal to noise and acquisition times to approximately
1 cm³, the impressive results that have been obtained in this
study suggest that it provides complementary information
to other imaging modalities. Future clinical studies should
be designed to include the acquisition of metabolic imaging
data in order to determine whether these results hold for a
larger cohort of patients.

Conclusions

This study supports the hypothesis that levels of choline,
creatine, NAA, lactate and lipid change in response to treat-
ment and that metrics describing these characteristics are
associated with survival. Integrating 3D H-1 MRSI into the
serial assessment of patients with GBM who are receiving
therapies that include anti-angiogenic agents may therefore
be helpful for resolving ambiguities caused by non-specific
treatment effects and for predicting outcome.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict
of interest.

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