MR Imaging–Based Analysis of Glioblastoma Multiforme: Estimation of IDH1 Mutation Status


ABSTRACT

BACKGROUND AND PURPOSE: Glioblastoma multiforme is highly aggressive and the most common type of primary malignant brain tumor in adults. Imaging biomarkers may provide prognostic information for patients with this condition. Patients with glioma with isocitrate dehydrogenase 1 (IDH1) mutations have a better clinical outcome than those without such mutations. Our purpose was to investigate whether the IDH1 mutation status in glioblastoma multiforme can be predicted by using MR imaging.

MATERIALS AND METHODS: We retrospectively studied 55 patients with glioblastoma multiforme with wild type IDH1 and 11 patients with mutant IDH1. Absolute tumor blood flow and relative tumor blood flow within the enhancing portion of each tumor were measured by using arterial spin-labeling data. In addition, the maximum necrosis area, the percentage of cross-sectional necrosis area inside the enhancing lesions, and the minimum and mean apparent diffusion coefficients were obtained from contrast-enhanced T1-weighted images and diffusion-weighted imaging data. Each of the 6 parameters was compared between patients with wild type IDH1 and mutant IDH1 by using the Mann-Whitney U test. The performance in discriminating between the 2 entities was evaluated by using receiver operating characteristic analysis.

RESULTS: Absolute tumor blood flow, relative tumor blood flow, necrosis area, and percentage of cross-sectional necrosis area inside the enhancing lesion were significantly higher in patients with wild type IDH1 than in those with mutant IDH1 (P < .05 each). In contrast, no significant difference was found in the ADCminimum and ADCmean. The area under the curve for absolute tumor blood flow, relative tumor blood flow, percentage of cross-sectional necrosis area inside the enhancing lesion, and necrosis area were 0.850, 0.873, 0.739, and 0.772, respectively.

CONCLUSIONS: Tumor blood flow and necrosis area calculated from MR imaging are useful for predicting the IDH1 mutation status.

ABBREVIATIONS: ASL = arterial spin-labeling; xTBF = absolute tumor blood flow; AUC = area under the curve; GBM = glioblastoma multiforme; IDH1 = isocitrate dehydrogenase 1; IDH1m = mutant IDH1; IDH1w = wild type IDH1; MGMT = O6-methylguanine-DNA methyltransferase; SNEC = percentage of cross-sectional necrosis area inside the enhancing lesion; NECarea = necrosis area; rTBF = relative tumor blood flow; TBF = tumor blood flow.

Glioblastoma multiforme (GBM) is highly aggressive and the most common type of primary malignant brain tumor in adults. The characteristic histologic appearance of GBM includes hypercellularity, nuclear polymorphism, high mitotic activity, prominent microvascular proliferation, and/or necrosis. MR imaging is the main noninvasive technique for diagnosing GBM. Conventional MR imaging techniques including pre- and post-contrast T1WI show precise anatomic localization and/or centrally nonenhancing regions, which are typically related histologically to necrotic areas. Diehn et al1 provided evidence that the amount of necrosis correlated with outcome in patients with GBM. In addition, correlations were recently identified between the prognosis of patients with GBM and several functional imaging parameters, including ADC derived from DWI, tumor blood volume calculated from DSC, and tumor blood flow (TBF) calculated from arterial spin-labeling (ASL) perfusion MR imaging.2–7 ASL is a recently developed MR perfusion imaging technique that has advantages of being noninvasive, not requiring an extrinsic tracer, and allowing reliable absolute quantification, which is not affected by a disrupted blood-brain barrier.8 ASL is increasingly...
recognized as a noninvasive method for quantitative CBF measurement for assessing stroke, neurodegenerative diseases, and brain tumors. ADC measurement is a widely used method. Good correlations have been reported between ADC and tumor cellularity, and its utility for application in glioma grading has been addressed in many studies.

GBMs are classified into primary and secondary GBMs. Primary GBMs develop rapidly de novo, without clinical or histologic evidence of a less malignant precursor lesion. In contrast, secondary GBMs develop by progressing from a low-grade diffuse astrocytoma or anaplastic astrocytoma. These GBM subtypes are usually indistinguishable histologically. However, genetic evidence suggests that mutations in isocitrate dehydrogenase (IDH1) can be used to identify most secondary GBMs. The IDH1 mutation status is an independent prognostic factor in patients with gliomas. In previous reports, patients with gliomas with IDH1 mutations had a better clinical outcome (median overall survival = 2.0–3.8 years) than those without such mutations (median overall survival = 0.8–1.1 years). In addition, a specific compound impairs the growth of mutant IDH1 but not wild type IDH1 glioma cells. These approaches may offer new possibilities for targeted therapy. The status of O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation is also an important factor for the prognosis of patients with GBM. Patients with GBM with MGMT promoter methylation are more responsive to temozolomide therapy and have better clinical outcome than those without it. Therefore, the detection of IDH1 mutations and MGMT promoter methylation is of great importance for patients with GBM. Carrillo et al suggested that patients with mutant IDH1 have low vascular endothelial growth factor levels, which are associated with contrast enhancement. These findings led to the hypothesis that measurement of tumor vascularity and the necrosis area would be helpful to differentiate IDH1 mutation status.

Our purpose was to investigate whether the IDH1 mutation and MGMT methylation status in GBM can be predicted by using MR imaging.

MATERIALS AND METHODS
This study was approved by the institutional review board of Kyushu University Hospital. Informed consent for study participation was waived due to the retrospective nature of this study.

MR imaging data of consecutive patients between May 2007 and August 2013 were obtained and retrospectively analyzed. Considering the effect of perfusion parameters, we excluded enrolled patients who received bevacizumab. Consequently, we examined data for 55 patients with GBM (54 primary and 1 recurrent) with wild type IDH1 (IDH1w: mean age, 54.8 ± 18.6 years; range, 5–83 years) and 11 patients with GBM (5 primary and 6 recurrent) with mutant IDH1 (IDH1m: mean age, 39.9 ± 11.8 years; range, 26–62 years). Among them, ASL was performed in 61.8% (34/55) of patients with IDH1w and 81.8% (9/11) of those with IDH1m. DWI was performed in 98.1% (54/55) of those with IDH1w and 100% (11/11) of those with IDH1m, and conventional MR imaging was performed in 100% (55/55) of those with IDH1w and 100% (11/11) of those with IDH1m. All primary and recurrent GBMs were histopathologically diagnosed by board-certified neuropathologists. The average interval between MR imaging and the operation was 7.1 days (range, 0–15 days).

MR Imaging
All images were obtained by using a 3T MR imaging unit (Achieva 3T TX; Philips Healthcare, Best, the Netherlands) and an 8-channel head array receiving coil for sensitivity encoding parallel imaging.

ASL
ASL was performed by using quantitative signal targeting with alternating radiofrequency labeling of the arterial region, a pulsed ASL technique developed by Petersen et al. The details of the sequence have been described elsewhere. Our quantitative signal targeting with alternating radiofrequency labeling of the arterial region protocol consisted of 84 dynamic or 42 pairs of labeled and nonlabeled image acquisitions. Of these, 24 pairs were acquired with crusher gradients (velocity-encoding threshold = 4 cm/s) and 12 pairs were acquired without crushers. These 36 pairs were acquired at a flip angle of 35°. An additional 6 pairs were acquired at a lower flip angle (11.7°) without crushers to estimate the actual flip angle that might vary across the brain due to inhomogeneity of B1. Other imaging parameters were as follows: labeling slab thickness = 150 mm, gap between the labeling and imaging slabs = 15 mm, sensitivity encoding factor = 2.5, TR/TE = 4000/22 ms, sampling interval = 500 ms, sampling time points = 13, FOV = 240 mm, matrix size = 64 × 64, imaging time = 5 minutes 52 seconds. Seven 6-mm-thick transverse sections (gap = 2 mm) were placed to cover the tumor.

DWI
DWI was performed by using a single-shot spin-echo echo-planar sequence with the following parameters: TR/TE = 3421/62 ms, 90° flip angle, NEX = 1, 22 transverse sections, sensitivity encoding factor = 2.5, section thickness/gap = 5/1 mm, FOV = 230 mm, 126 × 160 matrix, imaging time = 44.5 seconds. Diffusion sensitizing gradients were applied sequentially in the x, y, and z directions with b factors of 0 and 1000 s/mm².

Conventional MR Imaging
Postcontrast transverse T1-weighted spin-echo images (TR/TE = 400/10 ms, flip angle = 75°, NEX = 1, 22 sections, section thickness/gap = 5/1 mm, FOV = 230 mm, 256 × 173 matrix, imaging time = 2 minutes 43 seconds) were obtained. A standard dose (0.1 mmol/kg body weight) of a gadolinium-based contrast agent, gadopentate dimeglumine (Magnevist; Bayer HealthCare Pharmaceuticals, Wayne, New Jersey), gadoteridol (ProHance; Bracco Diagnostics, Princeton, New Jersey), or gadodiamide (Omniscan; GE Healthcare, Piscataway, New Jersey) was injected intravenously. Precontrast T1-weighted spin-echo, T2-weighted turbo spin-echo, and fluid-attenuated inversion recovery images were also obtained.

Detection of IDH1 Mutations and MGMT Promoter Methylation in Glioblastoma Tissues
GBM samples were obtained from each patient during the operation at our hospital. A portion of the tumor tissue was snap-
frozen in liquid nitrogen and stored at −80°C. Tumor DNA was isolated from the frozen blocks by using a QIAamp DNA Blood Mini Kit (QIAGEN, Tokyo, Japan). A 129-bp fragment spanning the catalytic domain of IDH1 including codon 132 was amplified by using the sense primer IDH1f 5′-CGGTCTTCAGAGCATT-3′ and the antisense primer IDH1r 5′-GCAAAATC-ACATTATTGCCAAC-3′, as described previously. Sequences were determined by using an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, California).

DNA methylation status of the MGMT promotor was determined by bisulfite modification and subsequent methylation-specific polymerase chain reactions. Methylation-specific polymerase chain reactions were performed by using the primers previously reported by Esteller et al. and 50-ng bisulfite-modified tumor DNA, in addition to both methylated and unmethylated control samples (CpGenome Universal Methylated and Unmethylated DNA; EMD Millipore, Billerica, Massachusetts). The polymerase chain reaction conditions included 35 cycles of 30 seconds each at 95°C, 60°C, and 72°C. The polymerase chain reaction products were electrophoresed on 3% agarose gels. The method has been described in detail before.

**Image Analysis**

The ASL data were analyzed on a desktop computer (Let’s note, Panasonic Corporation, Osaka, Japan). Maps of CBF were obtained by using dedicated software running on Interactive Data Language (Research Systems, Boulder, Colorado), which was developed and provided by Petersen et al. (National Neuroscience Institute, Singapore). Measurement of blood flow was performed by using ROI analysis by 2 independent neuroradiologists (K. Yamashita and O.T.), who were blinded to the clinical and pathologic information. A free software package (MRicro, http://www.mccauslandcenter.sc.edu/mricro/mricro/micro.html) was used to draw ROIs on the CBF maps. For each tumor, mean absolute (aTBF) and relative tumor blood flow (rTBF) were measured in each ROI (Fig 1A). Interrater agreement was evaluated by the
Each of the 6 parameters (aTBF, rTBF, ADCminimum, ADCmean, NECarea, and %NEC) was compared between patients with IDH1w and IDH1m and between patients with a methylated MGMT promoter and those with an unmethylated MGMT promoter by using the Student t test. A P value < .05 was statistically significant. The performance in discriminating between patients with IDH1w and IDH1m was evaluated by using receiver operating characteristic analysis. Area under the curve (AUC) values for the discrimination were calculated for parameters that were statistically significant. Multivariate logistic regression analysis was performed to evaluate the combination of the parameters. AUC values were compared with each other by using a nonparametric approach. All statistical analyses were performed by using JMP 11 Pro software (SAS Institute, Cary, North Carolina).

RESULTS

aTBF, rTBF, NECarea, and %NEC were significantly higher in patients with IDH1w (mean aTBF = 107.2 ± 58.7 mL/100 g/min, mean rTBF = 2.53 ± 1.05, mean NECarea = 557 ± 508 mm², and mean %NEC = 35.9% ± 21.2%) than in those with IDH1m (mean aTBF = 53.7 ± 24.8 mL/100 g/min, mean rTBF = 1.29 ± 0.51, mean NECarea = 138 ± 218 mm², and mean %NEC = 17.4% ± 20.2%) (P < .05 each, Fig 2). In contrast, no significant differences were found in ADCminimum (ADCminimum = 0.86 ± 0.18 × 10⁻³ mm²/s; range, 0.54–1.33 × 10⁻³ mm²/s in IDH1w, 0.92 ± 0.24 × 10⁻³ mm²/s; range, 0.61–1.30 × 10⁻³ mm²/s in IDH1m) and ADCmean (ADCmean = 0.97 ± 0.20 × 10⁻³ mm²/s; range, 0.59–1.46 × 10⁻³ mm²/s in IDH1w, 0.96 ± 0.21 × 10⁻³ mm²/s; range, 0.69–1.30 × 10⁻³ mm²/s in IDH1m) (P > .05 each).

No significant differences were observed in any parameters between patients with a methylated MGMT promoter and those with an unmethylated MGMT promoter (Table).

The optimal cutoff value was 70.0 mL/100 g/min for aTBF with 76.5% sensitivity, 88.9% specificity, and 79.1% accuracy. For rTBF, the optimal cutoff value was 1.55 with 88.2% sensitivity, 77.8% specificity, and 86.0% accuracy. For %NEC, the optimal cutoff value was 22.5 with 72.7% sensitivity, 81.8% specificity, and 74.2% accuracy. For NECarea, the optimal cutoff value was 151 mm² with 72.7% sensitivity, 81.8% specificity, and 74.2% accuracy. The AUCs for aTBF, rTBF, %NEC, and NECarea were 0.850, 0.873, 0.739, and 0.772, respectively (Fig 3). No significant difference in AUC values was found among aTBF, rTBF, %NEC, and NECarea. The combination of the 4 parameters increased the diagnostic performance (AUC = 0.915). The AUC value was sig-

FIG 2. Plots of aTBF (A), rTBF (B), ADCminimum (C), ADCmean (D), NECarea (E), and %NEC (F) in patients with IDH1w and IDH1m. The aTBF, rTBF, NECarea, and %NEC were significantly higher in patients with IDH1w compared with those with IDH1m (P < .05 each). In contrast, no significant difference was found in the ADCminimum and ADCmean.
Comparison between 6 parameters and MGMT methylation status

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Methylated</th>
<th>Unmethylated</th>
<th>P Value</th>
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<tbody>
<tr>
<td>aTBF (mL/100 g/min)</td>
<td>100.4 ± 11.3 (n = 19)</td>
<td>99.4 ± 55.4 (n = 15)</td>
<td>.96</td>
</tr>
<tr>
<td>rTBF</td>
<td>2.54 ± 13.1 (n = 19)</td>
<td>2.35 ± 0.81 (n = 15)</td>
<td>.62</td>
</tr>
<tr>
<td>ADCminimum (×10³/mm²/s)</td>
<td>0.88 ± 0.19 (n = 24)</td>
<td>0.84 ± 0.20 (n = 21)</td>
<td>.57</td>
</tr>
<tr>
<td>ADCmean (×10³/mm²/s)</td>
<td>0.97 ± 0.19 (n = 24)</td>
<td>0.96 ± 0.21 (n = 21)</td>
<td>.92</td>
</tr>
<tr>
<td>NECarea (mm²)</td>
<td>503 ± 424 (n = 25)</td>
<td>621 ± 430 (n = 21)</td>
<td>.36</td>
</tr>
<tr>
<td>%NEC</td>
<td>37.3 ± 211 (n = 25)</td>
<td>38.6 ± 22.7 (n = 21)</td>
<td>.85</td>
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95% limits of agreement in differences versus the average of the rTBF values, which ranged from −2.12 to 2.18 (Fig 4). The intraclass correlation coefficient was 0.861 (95% confidence interval, 0.743–0.925) for aTBF and 0.745 (95% confidence interval, 0.530–0.862) for rTBF, which indicated a high correlation. For the Spearman rank correlation coefficient, good correlation was shown for both aTBF (ρ = 0.774, P < .01) and rTBF (ρ = 0.709, P < .01) for the values between the 2 neuroradiologists.

Figures 5 and 6 show representative cases of IDH1w and IDH1m, respectively.

DISCUSSION

Our study demonstrated that both aTBF and rTBF were significantly higher in patients with IDH1w than in those with IDH1m. Microvascular proliferation is induced by the vascular endothelial growth factor, which shows markedly higher expression in primary than secondary GBMs. Diehn et al1 suggested that vascular endothelial growth factor production is associated with angiogenesis and contrast enhancement. The relationship between vascular endothelial growth factor and IDH1 remains uncertain. However, these results suggested that a correlation may exist between tumor vascularity and IDH1 mutation status. In addition, a previous study by using ASL showed that high TBF in GBM is associated with poor overall survival. ASL measurements may provide additional prognostic information.

In this study, both NECarea and %NEC were significantly higher in patients with IDH1w than in those with IDH1m. In GBM, hypoxia-mediated activation of the coagulation system causes intravascular thrombosis, which further increases intratumoral hypoxia and leads to abnormal endothelial cell proliferation and tumor necrosis. Previous studies demonstrated that large areas of ischemic and/or pseudopalisading necrosis are more frequent in primary than in secondary GBMs, and in patients with IDH1w than in those with IDH1m. Carlson et al25 indicated that necrosis is associated with higher levels of vascular endothelial growth factor. Our results are in line with these previous reports.

We found that both TBF and the necrosis area in patients with IDH1w were significantly higher than in those with IDH1m. The AUC value was significantly higher with the combination of all 4 parameters (aTBF, rTBF, NECarea, and %NEC) than with NECarea or %NEC alone. This is the first report to compare the performance of ASL, DWI, and gadolinium T1WI for predicting the IDH1 mutation status in GBM, to our knowledge. Our results suggested that the combination of TBF derived from ASL and measurement of the necrosis area may be a surrogate marker for predicting the IDH1 mutation status. Noninvasive estimates of tumor vascularity (aTBF, rTBF) and necrosis (NECarea, %NEC) may be useful for evaluating the prognosis of patients with GBM and their IDH1 mutation status. Patients with IDH1w and IDH1m follow different clinical courses, and GBMs with these mutations are considered to be 2 distinct disease entities. TBF and tumor necrosis area measurements play supportive roles as predictors of
the response to current treatment and tumor aggressiveness. These measurements may provide important information for selecting more or less intensive treatment.

With ADC measurement, no significant difference was found between patients with *IDH1w* and those with *IDH1m* in our study. Lee et al. showed that the mean ADC value in patients with *IDH1m* was significantly higher than that in those with *IDH1w*. This difference may be attributed to patient selection. The *IDH1m* group had a significantly higher proportion of anaplastic astrocytoma than the *IDH1w* group in their study. In our study, only patients with GBM were included. Lazovic et al. found no significant differences in ADC in nonnecrotic tumor regions between patients with *IDH1w* and those with *IDH1m*. On the basis of a radiologic-pathologic correlation study, no significant correlation between the Ki-67 labeling index and minimum ADC was noted for the GBM group. Our results are consistent with those in the literature.

*IDH1m* and *MGMT* promoter methylation are related to a better clinical prognosis. Patients with GBM with *MGMT* promoter methylation are more sensitive to temozolomide therapy and are associated with a favorable outcome. Noninvasive prediction of *IDH1* mutation and *MGMT* promoter methylation could contribute to the development of treatment strategies such as further targeted therapy. No significant differences were observed in any parameters derived from MR imaging between patients with a methylated *MGMT* promoter and those with an unmethylated *MGMT* promoter. Carrillo et al. indicated that the methylation status does not correlate with any imaging features (size, enhancement, noncontrast enhancing tumor, necrosis, edema, cysts, and location). The group of patients with an unmethylated *MGMT* promoter showed a significant difference in mean rCBV between pseudo-progression and real progression, though the group with a methylated *MGMT* promoter showed no significant difference in another study. These results suggest that predicting *MGMT* promoter methylation status from MR imaging may be challenging.

*IDH1* has been proved to delay glioma growth. Patients with GBM with *MGMT* promoter methylation are more sensitive to temozolomide therapy and are associated with a favorable outcome. Noninvasive prediction of *IDH1* mutation and *MGMT* promoter methylation could contribute to the development of treatment strategies such as further targeted therapy. No significant differences were observed in any parameters derived from MR imaging between patients with a methylated *MGMT* promoter and those with an unmethylated *MGMT* promoter.
Our study has some limitations. First, as mentioned earlier, not all patients were studied with all 3 imaging modalities (ASL, DWI, and postcontrast T1WI). Some recurrent cases of IDH1w and IDH1m were included in our study. The tumor sample was not acquired stereotactically before resection. However, a 3D MR image overlay navigation system and 5-aminolevulinic acid fluorescence-guided surgery were used to avoid necrotic or nonenhancing tumor regions when obtaining the GBM sample. Finally, automated MR imaging volumetric quantification of tumor necrosis was not applied because we believe that both pre- and postcontrast T1WI are required to correctly determine the enhancing area.

CONCLUSIONS
Our results suggested that TBF calculated from ASL and tumor necrosis area derived from conventional MR imaging are useful for predicting the IDH1 mutation status.

Disclosures: Koji Yamashita—RELATED: Grant: Japan Society for the Promotion of Science (Japanese grant) KAKENHI for UNRELATED: Science (Japanese grant) KAKENHI 26461828. Akio Hiwatashi—Disclosures: Koji Yamashita—Disclosures: for predicting the necrosis area derived from conventional MR imaging are useful.

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