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CASE REPORT

Prolonged and severe thrombocytopenia with pancytopenia induced by radiation-combined temozolomide therapy in a patient with newly diagnosed glioblastoma—analysis of O^6-methylguanine-DNA methyltransferase status

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Abstract We report a case of a 51-year-old woman with newly diagnosed glioblastoma multiforme (GBM) who was treated with surgery followed by the standard concomitant temozolomide (TMZ) and radiotherapy (RT). Although TMZ is generally safe and well-tolerated, she developed a sudden onset of prolonged and severe thrombocytopenia as the most prominent event of pancytopenia during the combined treatment, leading to discontinuation of the combined therapy. Thrombocytopenia lasted for more than 2 months with intensive, intermittent platelet transfusions. A bone marrow aspiration and biopsy performed after recovery of severe suppression still revealed reduced number of megakaryocytes. O^6-methylguanine-DNA methyltransferase (MGMT) analyses showed methylated MGMT promoter in GBM, but unmethylated promoters in both peripheral blood leukocytes and bone marrow cells. This is the first report suggesting the irrelevance of MGMT status of normal hematopoietic cells to TMZ-induced severe thrombocytopenia and pancytopenia.

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Keywords Temozolomide · Pancytopenia · Thrombocytopenia · Glioblastoma · O^6-methylguanine-DNA methyltransferase · Combined radiochemotherapy

Introduction

Temozolomide (TMZ) has been considered as the current standard chemotherapeutic agent in combination with radiotherapy (RT) for management of patients with glioblastoma multiforme (GBM) [1]. TMZ is generally safe and well-tolerated at continuous daily dose of 75 mg/m^2 during external beam radiotherapy for total 60 Gy, fractionatedly delivered in 6 weeks. The incidence of grade 3 or 4 myelosuppression, the dose-limiting toxicity, is relatively uncommon, being reported to occur in only 4% of patients [1]. TMZ is an alkylating agent giving rise to methylation at the O^6-position of guanine in DNA, thereby exerts antitumor effects in tumor cells. However, this lesion is effectively repaired by a DNA repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT) and a close relationship between MGMT status and sensitivity to TMZ has been demonstrated in GBM [2]. Here we report a case of GBM treated with the standard TMZ and RT regimen, who developed a sudden onset of prolonged and severe thrombocytopenia as the most prominent event of pancytopenia during the treatment, together with an analysis of MGMT status in hematological system.

Case report

A 51-year-old Caucasian woman with an unremarkable past medical history presented with a sudden onset of

features suggestive of raised intracranial pressure without any focal neurological deficits. Brain CT and MRI showed a well-enhancing mass lesion in the left frontal lobe with considerable perifocal brain edema which compressed lateral ventricles. There was a vague enhancement in the anterior part of corpus callosum suggesting infiltration of the tumor (Fig. 1a, b). She underwent a craniotomy and gross total removal of main mass in the left frontal lobe. The corpus callosum lesion was left untouched (Fig. 1c, d). Histopathologically the tumor was diagnosed GBM, WHO grade IV (Fig. 2a–d).

Postoperatively, the patient recovered well without developing any neurological sequelae. She received external beam X-ray irradiation therapy (2 Gy/fraction/day) 2 weeks after surgery, and was administered concomitantly with TMZ 140 mg/body/day (75 mg/m^2/day) daily. Her blood laboratory data at the time of radiochemotherapy initiation were all within normal ranges. She was then discharged from hospital and continued the therapy at the outpatient clinic.

Although her platelet counts remained 284 × 10^9/l on the treatment day 20, it rapidly fell down to 47 × 10^9/l a week after (day 27). TMZ was immediately discontinued (total TMZ dose was 27 × 140 mg = 3,780 mg) and she was readmitted to our hospital next day. But platelet level continued to decline, being 20 × 10^9/l on day 29, when she was started on blood products (platelet transfusion). Her platelet counts remained persistently low, thus she received serial platelet transfusions (total number of transfusions was 21) mostly twice a week until the last transfusion on day 92 (Fig. 3). Leukocytes and neutrophils also decreased gradually. Total leukocyte count (TLC) and neutrophils fell to 1.6 × 10^9/l and 0.72 × 10^9/l, respectively, on day 37, thereby he was started on growth factors (lenograstim 100 μg sc daily). Radiotherapy was also discontinued after

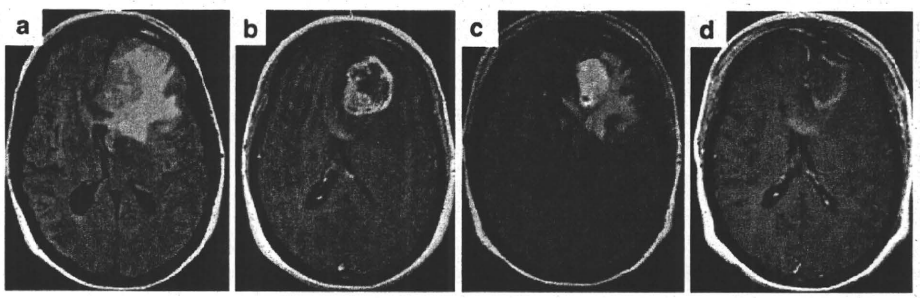

Fig. 1 **a, b** FLAIR (**a**) and T1-weighted MRI of brain with gadolinium (Gd)-contrast (**b**) axial sections showing a left frontal mass and an anterior corpus callosum lesion with surrounding edema. **c, d** On postoperative day 2, left frontal tumor was gross-totally resected but the corpus callosal lesion remained. **c** FLAIR image. **d** T1-weighted MRI with Gd

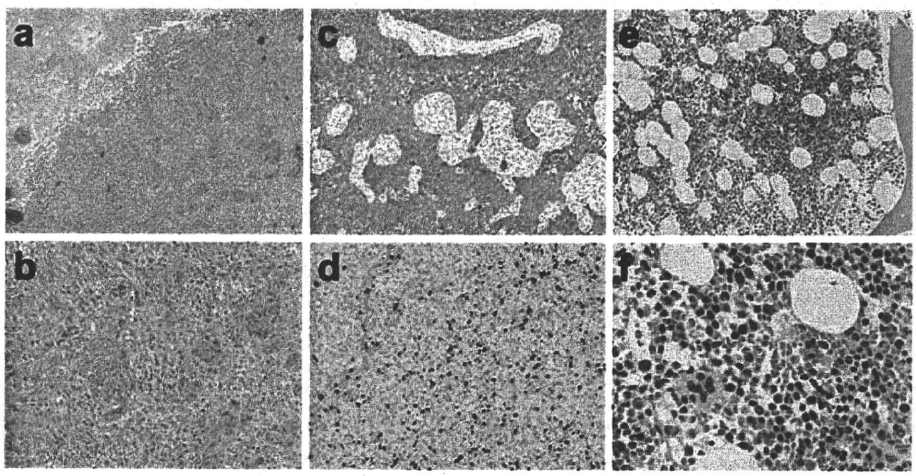

Fig. 2 Pathological findings. **a, b** Photomicrographs showing frontal lobe tumor with marked endovascular proliferation and necrosis, consistent with glioblastoma. **a** At a low magnification. **b** At a high magnification. **c** Immunohistochemistry staining with glial fibrillary acidic protein (GFAP), positive for tumor cells, but negative for vascular regions. **d** Ki-67 Immunostaining showing a high proliferative activity (~30% positivity). **e, f** Photomicrographs of bone marrow aspirated on postoperative day 137 (in a recovering phase after myelosuppression) demonstrating almost normal cellular marrow consisting of both erythroid and myeloid cells. Note that megakaryocytes remain hypoplastic. **e** At a low magnification. **f** At a high magnification

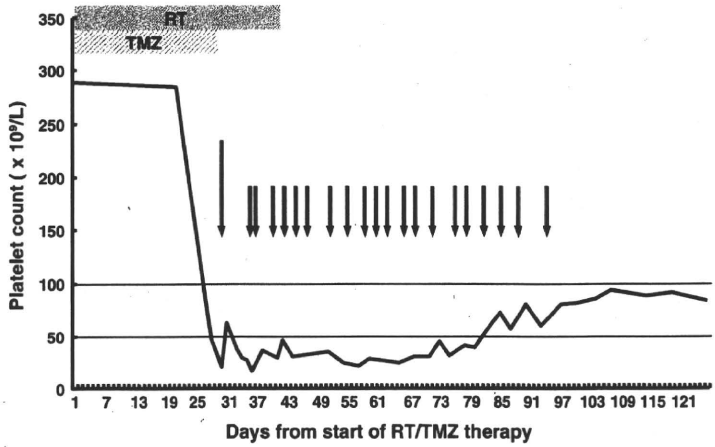

Fig. 3 Platelet counts of the present case through the course of serious thrombocytopenia following concomitant daily TMZ and RT. Periods of TMZ (*hatched*) and RT (*shaded*) administration are indicated on the top of the panel. Both treatments were discontinued in the middle of treatment schedule due to development of high grade myelosuppression. The upper horizontal line (at platelet count 100×10^9/l) indicates the level below which TMZ is no longer administered. The lower horizontal line (at platelet count 50×10^9/l) indicates the level below which patients are classified as having grade 3–4 thrombocytopenia. An *arrow* indicates the days when platelet transfusion was performed (total 21 transfusions). The *long arrow* illustrates a doubled dose

total dose of 52 Gy/26 fractions. However, TLC continued declining and it was 0.7×10^9/l with neutrophils at 0.017×10^9/l on day 43. Lenograstim dose was doubled (200 μg/day) since day 44, and prophylactic antibiotics (levofloxacin 600 mg/day) was started from day 46 (till day 65). The last growth factors were given on day 73. Hemoglobin (Hb) level decreased as well, and red blood cell transfusion was started on day 43 when Hb fell to 7.4 g/dl. Total number of red blood cell transfusions was 8; the last on day 79.

Gradually her hematological conditions recovered and remain at Hb 9.3 g/dl, platelet 92×10^9/l, TLC 2.6×10^9/l, and neutrophils 1.42×10^9/l without blood products nor growth factors on day 118. She was discharged on day 119 with no apparent neurological deficits and systemic disorders.

Despite the serious hematological adverse events described above, head CT scans performed on day 68 revealed tumor progression. MR images with gadolinium clearly showed significant relapse of enhancing tumor arising from the anterior corpus callosum, extending into the contralateral frontal lobe. Fortunately she has not presented any neurological symptoms and signs yet until transfer to a hospital in U.S.A. on day 131.

She underwent bone aspiration and biopsy on day 116. The bone marrow (BM) biopsy examination disclosed hyperplastic marrow for myeloid and erythroid cells, whereas hypoplastic for megakaryocytes; less than 1/10 of lower limit of normal range (Fig. 2e, f), suggesting that both myeloid and erythroid cells are at recovery phases, but megakaryocytes have not sufficiently recovered from severe suppressive conditions yet.

To elucidate potential causes for the serious and prolonged myelosuppression, MGMT status was analyzed in the GBM, peripheral blood leukocytes (PBL), and BM cells by means of Western blot, methylation-specific polymerase-chain reaction (PCR) (MSP) [3, 4], and pyrosequencing analyses [5]. The MGMT promoter of the GBM sample was methylated accompanied with undetectable MGMT protein, whereas MGMT promoter regions of DNA in both PBL and BM cells were unmethylated as are in most normal cells (Figs. 4, 5).

Discussion

TMZ is now widely used as the standard chemotherapeutic agent against GBM since the phase 3 clinical trial conducted by the European Organisation for Research and Treatment of Cancer and the National Cancer Institute of Canada demonstrated that concomitant RT plus TMZ followed by six cycles of TMZ significantly prolonged survival at the hazard ratio of 65% compared with RT alone in patients with newly diagnosed GBM [1]. In addition, TMZ can be administered orally, and appeared to have an excellent safety profile, with only 3% of patients developing high grade thrombocytopenia in the study [1]. However, a recent report by Gerber et al. disclosed that grade 3–4 thrombocytopenia developed in 10 out of 52 (19%) patients with newly diagnosed GBM treated with the concomitant RT plus TMZ [6]. Two (4%) of them have required continued platelet transfusions over 6 months because of sustained severe thrombocytopenia [6]. Others have reported serious myelosuppression and aplastic

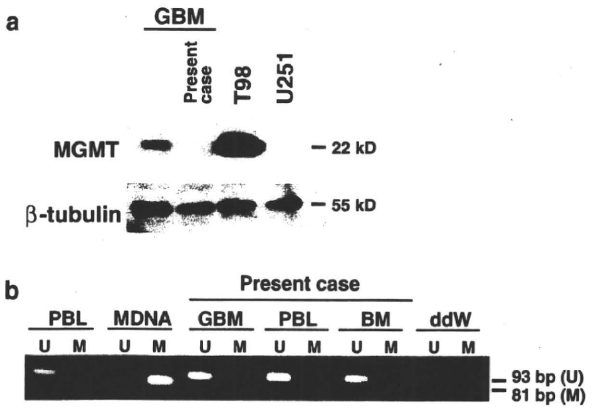

Fig. 4 MGMT analyses. **a** Western blot for protein expression of O^6-methylguanine-DNA methyltransferase (MGMT) in glioblastoma (GBM) specimens showing an undetectable level of MGMT expression in GBM of the patient. β-tubulin expression is shown as a loading control. Lysates of human glioma T98G and U251 cells were used as positive and negative controls for MGMT expression, respectively. **b** Methylation status of the *MGMT* promoter in biopsies from GBM, bone marrow (BM), and peripheral blood leukocytes (PBL) as determined by methylation-specific PCR [3], demonstrating that the *MGMT* promoter was methylated in GBM but unmethylated in PBL and BM. DNAs were subjected to bisulfite treatment that modifies unmethylated, but not methylated, cytosines to uracil. After purification, DNA was amplified using specific primers for the methylated (*M*) and modified unmethylated *MGMT* promoter (*U*), respectively. For DNA from bone marrow, PCR was performed in two reactions [4]. Normal PBL DNA (PBL) is negative for methylation, whereas human genomic DNA with enzymatic methylation (MDNA) was used as a positive control for methylation. The PCR products were separated on a 2.7% agarose gel. ddW designates a negative control for PCR reactions. The presence of a PCR product in the *U Lane* (93 bp) signifies the presence of unmethylated *MGMT* promoter, whereas a PCR product in the *M Lane* (81 bp) indicates the presence of methylated promoter. Patient materials were obtained with informed consent on approval from the Institutional Ethics Committees

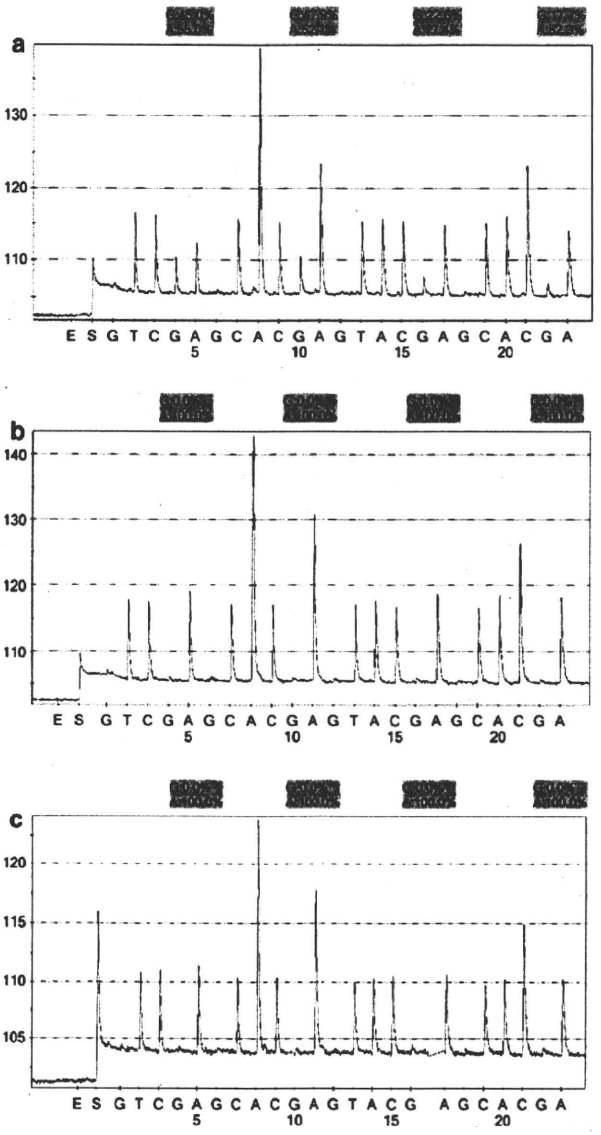

Fig. 5 Typical pyrograms obtained for the investigated DNA samples (only one of three programs is presented for each sample). Each box represents one of the four CpG positions interrogated by the pyrosequencing assay, starting on the left side with CpG 12, due to reverse sequencing the upper strand of the PCR product. As a consequence a C/TpG position appears as a CpG/A. The incorporation of the base guanine in this context represents the methylated fraction and the base adenine the unmethylated fraction, respectively. The tumor sample (**a**) shows methylated cytosine bases at each of the four CpG positions with a methylation score of 6.3, whereas DNA extracted from peripheral blood (**b**) and bone marrow (**c**) do not contain methylated MGMT sequences with methylation scores of −18.6 and −15.8, respectively [5]

anemia occurring after the concomitant RT plus TMZ therapy [7–9], suggesting that TMZ when used daily with RT may not always be safe enough as originally reported [1]. In the present case, there were no signs of appreciable adverse events until the 3rd week of RT plus TMZ therapy. On day 27 of the RT/TMZ therapy, an abrupt and severe drop of platelet emerged, followed by sustained pancytopenia. Predilection of this condition was not possible even her laboratory data were retrospectively reviewed.

Among blood corpuscles, platelet might be the preferential target of TMZ-mediated bone marrow toxicity. In the present patient, thrombocytopenia occurred as the first event, with the highest severity compared to neutropenia and anemia, and finally recovered after lasting more than 2 months with total 21 platelet transfusions (Fig. 3). Bone marrow examination performed after the recovery from pancytopenia revealed a significant decrease of megakaryocytes in contrast to normal distribution of both myeloid and erythroid cells (Fig. 2e, f). These findings suggest that platelet-lineage may be relatively more susceptible to TMZ in the hematopoietic system, in agreement with the report by Gerber et al. [6], although underlying mechanisms accounting for such selectivity need to be clarified.

Factors which potentially regulate sensitivity of cells to TMZ include MGMT, intact mismatch repair (MMR) system, cell-cycle progression regulatory proteins, and p53 function [10–12]. Among all, MGMT status has been strongly implicated in TMZ sensitivity based on the fact that MGMT effectively removes TMZ-induced methylation adducts at O^6-position of guanine in the tumor cell DNA, thereby repairing cytotoxic DNA damage without aid of any co-factors [11, 13]. *MGMT* gene expression is primarily regulated by methylation of its promoter as many other genes, but its promoter methylation is limited to tumor cells and is associated with promoter methylation of other cancer-associated genes, including *CDKN1A*, *MLH1* and *CDKN2A* [3]. Indeed, both the expression level of MGMT protein and methylation status of the promoter region of the *MGMT* gene have been demonstrated to significantly correlate with survival rates of patients with GBM after TMZ treatment [2, 14, 15]. Since TMZ is administered orally, it can induce DNA damage in any cells of systemic organs including bone marrow hematopoietic stem cells. *MGMT* is a house-keeping gene, so its promoter region is generally unmethylated and MGMT protein is expressed in normal cells. This may protect normal cells to be seriously damaged by DNA methylating agents such as TMZ. Alternatively, when MGMT activity is suppressed in hematopoietic cells, for instance in combination with MGMT depleting agent O^6-benzylguanine, TMZ treatment would increase a risk of severe myelosuppression [11], providing with a hypothesis that one might be predisposed to this condition by having suppressed MGMT activity or methylated MGMT promoter in hematopoietic cell system. This question was, however, never investigated in the previous reports describing TMZ-induced serious pancytopenia [6–9]. Here we demonstrated, for the first time, that the MGMT promoter regions of both PBL obtained before TMZ treatment and BM cells at the recovery phase from the myelosuppression were clearly unmethylated (Figs. 4b, 5) in the patient suffering TMZ-related prolonged thrombocytopenia with pancytopenia, although her GBM had the methylated MGMT promoter and its protein expression was scarcely detectable (Fig. 4a, b). These observations suggest that this serious toxicity was not attributable to the MGMT status in her normal hematopoietic cells.

Other possible predisposing factors for the prolonged myelosuppression might include anticonvulsants and H_2-blockers, both of which belong to the class of drugs that have been associated with thrombocytopenia [16], as well as trimethoprim-sulfamethoxazole for prevention from *Pneumocystis jirovecii* pneumonia [9, 17]. She was taking an H_2-blocker and trimethoprim-sulfamethoxazole at the onset of thrombocytopenia, but it is yet difficult to determine the degree to which they may have contributed to the myelosuppression, in agreement with reports by others [9].

We have not observed any prolonged, high grade myelosuppression by the standard 5-day on/23-day off TMZ regimen in nitrosourea-pretreated patients with recurrent GBM [14]. The extent of MGMT depletion differs by dose and schedule of TMZ administration [18]. Tolcher et al. demonstrated that a 21-days consecutive TMZ administration was superior in MGMT depletion compared to a 7 straight days regimen, suggesting that daily TMZ dosing concomitant with RT may potentially have a greater risk of inducing high grades of toxic effects on not only tumor cells, but also normal cells like hematopoietic cells.

We found early progression of the residual tumor after the RT/TMZ treatment, albeit the resected GBM carried the methylated MGMT promoter. Since GBM comprises heterogeneous cell populations, the recurrent lesion might have contained a higher proportion of MGMT-unmethylated cells than the resected tumor. Alternatively, the relapsed lesion with contrast enhancement may not have been recurrence, but pseudoprogression after the combined RT/TMZ therapy. GBMs with methylated MGMT promoter have been reported to undergo pseudoprogression at a significantly higher rate than those with unmethylated MGMT [19].

Although TMZ has been regarded as safe, the case presented here provides a rare incidence of serous hematological complications during low dose TMZ and RT. Clinicians should be aware of this potential risk, and further studies to investigate causative factors involved in this phenomenon need to be pursued for prediction of "high risk" patients.

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Standard Therapy for Glioblastoma— A Review of Where We Are

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Abstract

Glioblastoma is the most common primary malignant brain tumor in adults and is a challenging disease to treat. The current standard therapy includes maximal safe surgical resection, followed by a combination of radiation and chemotherapy with temozolomide. However, recurrence is quite common, so we continue to search for more effective treatments both for initial therapy and at the time of recurrence. This article will review the current standard of care and recent advances in therapy for newly-diagnosed and recurrent glioblastomas, based on the most authoritative guidelines, the National Cancer Institute's comprehensive cancer database Physician Data Query (PDQ®), and the National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology™ for central nervous system cancers (V.1.2010), to elucidate the current position and in what direction we are advancing.

Key words: glioblastoma, standard therapy, chemotherapy, temozolomide, clinical trial

Definition of Standard Therapy

Standard therapy is the treatment that experts agree is appropriate, accepted, and widely used, and is also called best practice and standard of care.[14] Health care providers are obligated to provide patients with standard therapy. Physicians are not allowed to provide patients with non-standard therapy without explaining the reason why standard therapy will not be provided and obtaining informed consent. Every clinical trial should have a convincing scientific basis to indicate that testing the treatment is worthwhile, and the patients should be informed that the test treatment is not a standard therapy, which is requisite from an ethical point of view. The Institutional Review Board (IRB) will examine the protocols, case report forms, and related documents from both scientific and ethical points of view. In randomized phase 3 studies, the control arms are always standard therapies of the diseases.

Standard Therapy for Glioblastoma in Physician Data Query (PDQ®)

PDQ® is the National Cancer Institute's comprehensive cancer database, and is the most authoritative guideline.[15] PDQ® is written in an itemized manner, so needs some commentary and explanations (Table 1).

Table 1 Standard therapy for glioblastoma in PDQ®

Surgery plus radiation therapy for elderly glioblastoma patients
No additional benefit from brachytherapy added to external-beam radiation therapy and carmustine (BCNU)
BCNU-impregnated polymer (Gliadel wafer) implanted during initial surgery
Radiation therapy and concurrent chemotherapy with temozolomide

PDQ®: Physician Data Query.

The first point is the treatment of glioblastoma (GBM) in the elderly population. Since the landmark European Organization for the Research and Treatment of Cancer (EORTC)/National Cancer Institute of Canada (NCIC) study published in 2005, the standard therapy for GBM has been post-operative adjuvant radiotherapy with concomitant and adjuvant temozolomide (TMZ) (so-called Stupp's regimen).[25] However, the patients eligible for this study were aged from 18 to 70 years, so the standard therapy for GBM patients aged over 70 years remains undetermined. Because the frequency of severe adverse events of TMZ is less than 10%, and the pharmacokinetic profile of TMZ is not age-dependent, investigators surmise that Stupp's regimen would be applicable for elderly patients, but this notion has not actually been proven yet.

A randomized phase 3 study comparing postoper-

ative supportive care and postoperative radiation therapy plus supportive care was performed for GBM patients over 70 years old.[10] The median survival time was 29.1 weeks for the 39 patients who received radiation therapy plus supportive care and 16.9 weeks for the 42 patients who received only supportive care. The hazard ratio of death in the radiation therapy arm was 0.47 (95% confidence interval [CI] 0.29–0.76; $p = 0.002$). This study was discontinued prematurely at the first interim analyses, because the radiotherapy plus supportive care arm was superior to the only supportive care arm with a preset boundary of efficacy. Post-operative radiotherapy resulted in a robust improvement in survival in elderly patients with GBM, and is now the standard therapy for this population. To prove that a full dose of 60 Gy/30 fractions was necessary for elderly GBM patients, a randomized study of patients 60 years and older comparing post-operative radiotherapy of 60 Gy/30 fractions (standard course) and 40 Gy/15 fractions administered over the course of 3 weeks (short course) was performed. Overall survival (OS) was similar for the two groups; 5.1 months for the standard course arm, and 5.6 months for the short course arm ($p = 0.57$).[22] Although there was concerns about the power of the study, which was discontinued prematurely at the first interim analysis when 100 patients were recruited, the results showed the outcomes of the two arms were statistically equivalent, so the short course of radiotherapy seemed to be the reasonable treatment option for elderly patients with GBM.

Deterioration of cognitive function is a well known adverse effect of radiotherapy, especially in the elderly population. Treatment with only TMZ, without radiotherapy, may be equivalent in OS and would provide better health-related quality of life (QoL), which is a reasonable hypothesis to be tested in elderly GBM patients. Three randomized phase 3 studies for elderly GBM patients are on-going: a three-arms study by the Nordic Clinical Brain Tumor Group assessing the efficacy of short course radiotherapy and only TMZ arms with standard course radiotherapy of 60 Gy; a study by the German Neuro-Oncology Working Group simply testing the efficacy of only TMZ treatment versus the standard course of radiotherapy, and the study by NCIC and EORTC aiming at the assessment of the additive effect of TMZ to short course radiotherapy. Three institutes from Japan, Kitano Hospital, Hiroshima University, and the International Medical Center, Saitama Medical University are members of the international study group for the NCIC/EORTC study, CE.6. Conclusions from these studies will decide if only TMZ is equivalent to radiotherapy, and provides better QoL, and if concomitant and adjuvant TMZ with short course radiotherapy would be valuable for elderly GBM patients.

The second point in PDQ® is evaluation of the efficacy of brachytherapy for GBM. A randomized cooperative study showed no additional benefit from brachytherapy added to external-beam radiation therapy and carmustine (BCNU) (NIH Trial 87-01).[23] Interstitial brachytherapy is one of the techniques to deliver high doses of irradiation to the tumor beds. Stereotactic radiotherapy is another high-dose local radiotherapy technique, which also failed to show survival advantage compared to external beam irradiation in a phase 2 study by the Radiation Therapy Oncology Group (RTOG 0023).[4] Because of the highly invasive nature of GBM, however high the irradiated dose is, the effect of radiotherapy would be limited as the irradiated field is restricted to the enhanced lesion.

The third point in PDQ® is the evaluation of BCNU-impregnated polymer (Gliadel® wafer) implanted during surgeries. A multicenter randomized double-blinded controlled trial with 240 patients with high-grade glioma including 207 GBM and 21 anaplastic glioma reported significantly longer OS for patients who had Gliadel® wafer placed intraoperatively (13.8 months for Gliadel® wafers vs. 11.6 months for placebo; HR 0.73, 95% CI 0.56–0.95; $p = 0.0018$).[32] However, a subanalysis of 207 GBM patients could not show significantly longer survival with Gliadel® wafer (13.1 months in the Gliadel®-treated group and 11.4 months in the placebo-treated group, $p = 0.08$). The spacial and temporal distribution of BCNU released from the polymer was calculated by a mathematical simulation model.[31] The penetration depth of BCNU from a polymer was estimated to be 0.5 cm. The penetration depth was defined as the average distance measured from the surface of a polymer at which the drug concentration is 1% compared to that of the polymer surface. The distance of penetration is short because BCNU has a high transvascular permeability and, therefore, is very easily absorbed into the systemic circulation. BCNU molecules, being lipid-soluble and very permeable, enter the bloodstream before they can travel far. Together with the short half life of BCNU (1.5 hours), the short distance of penetration would limit the efficacy of the therapy.

The last point in PDQ® is radiation therapy and concurrent chemotherapy. A randomized study performed by EORTC and NCIC was a landmark of GBM treatment. Radiation therapy plus TMZ followed by 6 months of adjuvant TMZ in patients with newly diagnosed GBM demonstrated a statistically significant survival advantage over simple

radiotherapy.[25] The median OS was 14.6 months with radiotherapy plus TMZ and 12.1 months with only radiotherapy (HR 0.63, 95% CI 0.52 to 0.75; $p < 0.001$). The treatment is relatively safe and well tolerated. The combined treatment regimen consists of concomitant and adjuvant TMZ with radiotherapy. While the dose of TMZ for adjuvant phase is 150–200 mg/m^2 for 5 days every 4 weeks, TMZ of 75 mg/m^2 is administered daily during the concomitant phase with radiotherapy. The rationale for the small dose and continuous administration of TMZ with radiotherapy are radiosensitization induced by TMZ and chemosensitization by O^6-methylguanine-deoxyribonucleic acid-methyltransferase (MGMT) depletion induced by TMZ. Radiosensitivity enhancement of tumor cells by TMZ was shown in vitro and in vivo. The enhancing effect involves inhibition of deoxyribonucleic acid (DNA) repair leading to increased mitotic catastrophe.[11] MGMT depletion induced by protracted TMZ schedules was shown in the peripheral blood mononuclear cells. MGMT decreased significantly after 7, 14, and 21 days of treatment with low-dose and protracted TMZ administration, thus resulting in autosensitization to TMZ.[27] MGMT is the key enzyme to determine the sensitivity for TMZ. In the clinical setting, promoter methylation of MGMT is one of the factors contributing to better outcomes for GBM patients treated by TMZ.[9]

In the combined treatment arm with TMZ and radiotherapy, patients with GBM with methylated MGMT promoters showed significantly better OS than those with unmethylated MGMT promoters.[9] In the only initial radiotherapy arm, patients with methylated MGMT promoter also showed improvement in OS. This was expected because more than 70% of the patients in the radiotherapy arm received chemotherapy, most likely with an alkylating agent at progression, and 60% of these patients received TMZ at progression. When progression-free survival (PFS) was analyzed, so eliminating second-line therapies as a confounding factor, PFS was only prolonged in patients with methylated MGMT promoters who were treated with radiotherapy plus TMZ. However, a close look at the Kaplan Meyer survival curves of patients in the radiotherapy arm with methylated or unmethylated MGMT promoters finds that the PFS of patients with methylated MGMT promoter showed slightly longer survival than that of patients with unmethylated MGMT promoter. In another set of patients who received radiotherapy without any alkylating agents, MGMT promoter methylation was predictive of response to radiotherapy and good prognosis.[21] Although the mechanism of radiation resistance by MGMT activation is not clear, methylated MGMT promoter may be a surrogate marker of as yet unidentified processes, other than TMZ resistance, that contribute to the overall aggressive biology of GBM. Furthermore, cancer-associated DNA methylation may affect the expression of many CpG island-associated genes including MGMT, either modifying sensitivity to radiation or resulting in less aggressive phenotype.[18]

Is ACNU Only a Memory?

In 2000, the Japan Clinical Oncology Group (JCOG) brain tumor study group was discussing how to initiate clinical studies for brain tumors in Japan. One of the foci of the discussion was the standard therapy for GBM at that time. 1-(4-Amino-2-methyl-5-pirimidinyl)-methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride (ACNU) was widely used based on a small randomized study in Japan in the 1980s.[26] The response rate was better in the combined treatment arm with ACNU and radiotherapy (47.5% in the combined treatment arm, 13.5% in the simple radiotherapy arm), but the OS was not significantly different (median OS was 14 months and 12 months, respectively). Although the combined therapy with ACNU and radiotherapy was promising, a randomized phase 3 study had not been performed. Nevertheless, without a phase 3 study, the combination of ACNU and radiotherapy was adapted as the standard therapy for malignant gliomas in Japan. The brain tumor study group of JCOG considered this as the community standard of GBM therapy that was made the starting point.

The JCOG 0305 study was a randomized phase 2 study comparing two combined-treatment protocols, ACNU with radiotherapy and procarbazine (PCZ) plus ACNU with radiotherapy. In the PCZ plus ACNU arm, PCZ was administered before ACNU aiming to deplete MGMT and to enhance the chemosensitivity to ACNU.[24] When the phase 2 study cleared a preset boundary of efficacy, the phase 3 study would begin, which was the original design. However, an interim analysis revealed there was no survival advantage of the test arm. This study was discontinued and did not proceed to phase 3. The control arm, ACNU with radiotherapy, achieved a median OS of 16.6 months for GBM, which should have been the basic data for the clinical trials for GBM thereafter in Japan. In 2005, the results of the combination of TMZ and radiotherapy were published as previously mentioned, and the combination of TMZ and radiotherapy became the standard therapy of GBM worldwide. No randomized comparison of ACNU and TMZ has been considered so far, based on the following reasoning.

The HR for death in the radiotherapy plus TMZ arm was 0.63 (95% CI 0.52–0.75; $p < 0.001$).[25] Meta-analysis evaluating the effectiveness of nitrosoureas (mainly BCNU) found the HR was 0.85 (95% CI 0.78–0.91; $p < 0.0001$). Considering the 95% CI, nitrosoureas were not thought to be as good as TMZ. The OS and PFS for GBM patients treated with concomitant and adjuvant TMZ with radiotherapy were 14.6 months and 6.9 months, respectively, whereas those with ACNU and radiotherapy were 16.6 months and 5.1 months based on the JCOG study, respectively. The better OS with ACNU and radiotherapy in Japan was possibly related to salvage therapies including repeat surgeries and stereotactic radiosurgeries, and to elaborate supportive care. The better OS with ACNU treatment was not due to better tumor control by ACNU because the PFSs were similar. Another retrospective report comparing BCNU and TMZ also showed that PFS was not significantly different between the two groups.[29] Severe adverse events (CTCAE grade 3 or higher) are more frequent in ACNU-treated patients than in TMZ-treated patients; leucopenia in 39% and 3% of the patients, respectively.

As the next step, the JCOG brain tumor study group has just started a randomized phase 2 study comparing a combination therapy of interferon-β plus TMZ with radiotherapy and a combination therapy of TMZ with radiotherapy, considering the latter as the standard therapy for GBM (JCOG 0911). This strategy is based on data showing the sensitizing effect of IFN-β for TMZ was possibly due to attenuation of MGMT expression via induction of the protein p53.[16]

When GBM Recurs

The National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology™ is available on-line (http://www.nccn.org). The guideline for central nervous system cancers (V.1.2010) states surgical resection should be considered first if recurrent or progressive tumors are resectable. Systemic chemotherapies are indicated if allowed by the performance status of the patients.

Bevacizumab with or without chemotherapy is the first in the list of possible second line chemotherapies for GBM (Table 2). As we learn more about the biology of GBM and its aberrant signaling pathways, the neuro-oncology community has begun to investigate the role of molecular targeted therapies. The angiogenesis pathways and their associated antiangiogenic agents are the most promising topic recently. Bevacizumab, a humanized monoclonal antibody that targets vascular endothelial growth factor (VEGF), was first approved in combination with chemotherapy for colorectal, lung, and breast cancers. Despite initial reluctance to evaluate bevacizumab in patients with brain tumors because of concerns with intracranial hemorrhage, the combination of bevacizumab and irinotecan was studied in a single-arm phase 2 study for recurrent GBM.[30] The response rate was 57%, and PFS at 6 months was 46%. These results compared quite favorably with historical data of response rate of 8% and PFS at 6 months of 21% by TMZ for recurrent GBM.[34] To clarify the contribution of irinotecan, a large phase 2 study randomized 167 patients with recurrent GBM to either single agent bevacizumab or bevacizumab plus irinotecan. The response rates were 28% in the single treatment arm with bevacizumab and 38% in the combination arm of bevacizumab plus irinotecan, and the PFS at 6 months was 43% and 50%, respectively.[8] Curiously enough, the randomized design of the trial was not designed to compare outcomes in the two treatment groups, but to evaluate their superiority to the historical results of salvage chemotherapies, 15% of PFS at 6 months, without bias in treatment assignment. Bevacizumab is usually well tolerated, with the most common adverse effects being hypertension and minor bleeding, such as epistaxis. Intracranial hemorrhage occurred in less than 4% of patients and was severe in only approximately 1% of patients.

Table 2 The second-line chemotherapies for recurrent glioblastoma in NCCN guideline

Bevacizumab with/without chemotherapy
Temozolomide
Nitrosourea or PCV
Cyclophsphamide
Platinum-based regimens

NCCN: National Comprehensive Cancer Network; PCV: procarbazine, lomustine, and vincristine.

Individual infiltrative tumor cells tend to grow along preestablished normal cerebral vasculature, so there is no need for tumor-associated angiogenesis from the tumor cells in the central core. Indeed, there is at least a theoretical concern that inhibiting malignant glioma angiogenesis may have little effect on the infiltrative component of the disease and so little impact on the overall survival of the patient. Furthermore, recent laboratory evidence suggests that inhibition of VEGF may actually increase the invasive nature of tumor cells.[19] There seems to be a proinvasive adaptation to anti-angiogenic therapy, as suggested by magnetic resonance imaging in a subset of GBM patients who developed multifocal

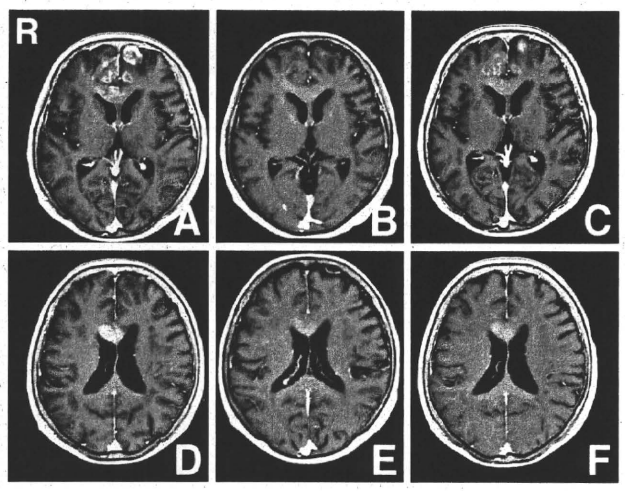

Fig. 1 Initial (A, D) and two follow-up magnetic resonance images 3 days (B, E) and 10 days (C, F) after bevacizumab treatment of a 68-year-old female patient with glioblastoma showing the heterogeneously enhanced tumor regressed 3 days after bevacizumab treatment, but reappeared 7 days later.

recurrence of tumors during the course of therapy with bevacizumab.[6,13,17] The infiltrative tumor cells are most often responsible for clinical relapse and ultimately the death of patients with gliomas. Early results from phase 2 trials showed that incorporation of bevacizumab into the standard initial treatment for newly diagnosed GBM increased median PFS, but prolongation of OS is still unclear. Two large phase 3 trials for newly diagnosed GBM are currently randomizing patients to standard radiotherapy and TMZ with or without bevacizumab.

A unique advantage of bevacizumab is the ability to decrease peritumoral edema. Patients treated with bevacizumab often have decreased corticosteroid dependence secondary to neutralization of VEGF, a known vascular permeability factor. Vascular permeability is decreased in and around the tumor, so decreasing both cerebral edema and the uptake of gadolinium within the tumor. An illustrative case (Fig. 1) showed marked decrease of enhancement on MRI after three days of bevacizumab administration. The decrease in enhancement was not due to tumor shrinkage as the enhancement was regained 7 days later (Fig. 1). As such, the remarkable radiographic response rates and PFS by bevacizumab should be interpreted cautiously.

A couple of successful regimens suggested low dose and continuous TMZ administration as a rechallenge was effective for recurrent disease.[20,33] Due to its usage as the first-line treatment of GBM, TMZ has been no longer considered by many investigators to be a reasonable choice for patients with recurrent GBM. However, alternative schedules of TMZ addressing different pathophysiological mechanisms could be effective even after progression during standard TMZ regimens.[33] There are several rationales supporting TMZ rechallenge. Firstly, there may be a benefit from alternative modes of action, such as antiangiogenic properties of a metronomic regimen. Secondly, as MGMT is inactivated after each reaction of removal of methyl bases (suicide enzyme), exposure to continuous and low-dose TMZ depletes MGMT activities. Thirdly, the schedule of temozolomide permits a greater drug exposure than the conventional schedule of 5 days every 28 days, with comparable or even lower toxicities.

A "one week on/one week off" scheme (150 mg/m^2 at days 1–7 and days 15–21, in a 28-day cycle) has been associated with considerable efficacy and was tolerated by patients. Another alternative is an intensified three out of four weeks approach (75–100 mg/m^2 at days 1–21, in a 28 day cycle). This regimen may yield similar results with respect to efficacy, but a higher rate of toxicity, specifically lymphopenia and infection, has been reported. Another regimen is a metronomic administration of TMZ, 20 mg daily.

The third optional treatment for recurrent GBM is regimens containing nitrosoureas, such as procarbazine, lomustine, and vincristine (PCV) chemotherapy. A randomized trial by the Medical Research Council Brain Tumour Working Party showed no benefit to PCV chemotherapy for newly diagnosed GBM.[12] However, PCV has certain activity, especially for malignant glioma with oligodendroglial component.[3,28] Therefore, this regimen may be important for recurrent GBM.

Cyclophosphamide is among the list of possible chemotherapies for recurrent GBM. However, the reference cited in the NCCN guideline is a report of recurrent anaplastic astrocytomas, and the efficacy of cyclophosphamide for recurrent GBM is not known.[5] Lastly, platinum-based regimens are reported to show modest activities for recurrent GBM.[1,2,7]

On-going Phase 3 Trials for Future Revision of the Standard Therapy

Table 3 shows four on-going randomized phase 3 confirmatory trials for GBM. Another schedule of TMZ administration (RTOG 0525), additive effect of bevacizumab (RTOG 0825, AVAglio), and also possible additive effect of cilengitide, integrin $\alpha v\beta 5$ inhi-

Table 3 Three on-going randomized phase 3 studies for newly diagnosed glioblastoma

Regimen	Organized by	Number of patients
Stupp's regiman with/without bevacizumab	Roche (AVAglio)	920
Stupp's regimen with/without bevacizumab	RTOG 0825	720
Stupp's regimen with/without cilengitide for patients with methylated MGMT promoter status	Merck Serono (CENTRIC)	504
Stupp's regimen vs. adjuvant dose-dense (3-1) temozolomide	RTOG 0525	834

MGMT: O^6-methylguanine-deoxyribonucleic acid-methyltransferase.

bitor, for MGMT promoter methylated GBM (CENTRIC) are to be tested for newly diagnosed patients. In conclusion, the survival of patients with GBM continues to improve, albeit more slowly than we would like. Various new agents are currently under study, singly and in combination. Improved understanding of the complex biology of GBM may allow for more rational and effective therapy selection for patients, further extending survival in the years to come.

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CLINICAL STUDY – PATIENT STUDY

Prediction of malignancy grading using computed tomography perfusion imaging in nonenhancing supratentorial gliomas

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Abstract Tumor grade differentiation is often difficult using routine neuroimaging alone. Computed tomography perfusion imaging (CTP) provides quantitative information on tumor vasculature that closely parallels the degree of tumor malignancy. This study examined whether CTP is useful for preoperatively predicting the grade of malignancy in glioma showing no enhancement on contrast-enhanced magnetic resonance imaging (MRI). Subjects comprised 17 patients with supratentorial glioma without enhancement on MRI. CTP was performed preoperatively, and absolute values and normalized ratios of parameters were calculated. Postoperatively, subjects were classified into two groups according to histological diagnosis of grade 3 (G3) glioma or grade 2 (G2) glioma. Absolute values and normalized ratios for each parameter were compared between G3 and G2. Accuracies of normalized ratios for cerebral blood flow (*n*CBF) and cerebral blood volume (*n*CBV) in predicting a diagnosis of G3 were assessed. In addition, *n*CBV was compared between diffuse astrocytoma, G2 oligodendroglial tumor (OT), and G3 OT. Values for *n*CBF and *n*CBV differed significantly between G3 and G2. Using *n*CBV of 1.6 as a cutoff, specificity and sensitivity for distinguishing G3 were 83.3% and 90.9%, respectively. No significant difference in *n*CBV was seen between diffuse astrocytoma and G2 OT, whereas differences were noted between G2 and G3 OTs, and between diffuse astrocytoma and G3 OT. CTP offers a useful method for differentiating between G3 and G2 in nonenhancing gliomas.

Keywords Computed tomography perfusion imaging · Diffuse astrocytoma · Glioma · Nonenhancement · Oligodendroglioma · Preoperative diagnosis

Introduction

Glioma is graded according to World Health Organization (WHO) classification, with grade 1 or 2 graded as low-grade glioma (LGG) and grade 3 or 4 commonly defined as high-grade glioma (HGG) [1]. As treatment and prognosis differ substantially between LGG and HGG, the ability to differentiate between grade 2 (G2) glioma and grade 3 (G3) glioma, as the border between LGG and HGG, is very important. On contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MRI), G2 gliomas are nonenhanced due to preservation of blood–brain barrier (BBB), whereas G3 gliomas are commonly enhanced due to increased vascular permeability caused by disruption of the BBB within the tumor [2–4]. However, the relationship between histological grading and contrast enhancement on CT and MRI is not always clear. Preoperatively differentiating between G3 and G2 gliomas that are nonenhanced on conventional neuroimaging is often difficult. When patients with nonenhancing glioma are encountered, neurooncologists may perform various examinations to differentiate between G3 and G2 gliomas, such as positron emission tomography (PET) for direct assessment of tumor

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metabolism, magnetic resonance spectroscopy to detect magnetic resonance signals of metabolites, and diffusion-weighted MRI to clarify structures within and surrounding the tumor. Assessment of intratumoral vasculature is one approach that may help to clarify the intratumoral biological characteristics and malignancy of a tumor, as intratumoral angiogenesis and high vascularity, which are regulated by hypoxia and various vascular endothelial growth factors, are essential for tumor growth and progression [5–7].

Angiography enables direct observation of intratumoral vessels, but is hazardous and remains limited for depiction of intratumoral microvasculature. Magnetic resonance perfusion imaging (MRP) and CT perfusion imaging (CTP) provide reliable information on the intratumoral microvasculature [8–12]. Numerous studies of perfusion imaging have shown that increasing malignancy of the glioma is associated with increased intratumoral blood volume and vascular permeability [10, 13–15]. Quantitative evaluation from perfusion imaging thus depends on both the microvasculature (vascular density and diameter), and vascular permeability due to disruption or absence of the BBB within the tumor. Previous reports have shown good correlations between findings on perfusion imaging and malignancy grading in enhancing glioma. In contrast, the BBB of vessels is preserved in nonenhancing glioma, since extravasation of contrast medium through the BBB in tumor vessels is considered to represent the main cause of tumor contrast enhancement [4]. As MRI remains the preferred technique for assessing brain tumors, studies using MRP to thoroughly evaluate gliomas greatly outnumber those using CTP, and MRP has also been applied to neurooncological applications for nonenhancing gliomas, such as determining biopsy targets and predicting malignant progression [16–18]. In recent years, CTP has gained acceptance as a valuable imaging technique for assessing hemodynamics in brain tumors [13, 14, 19–22]. However, whether CTP is useful for grading malignancy of nonenhancing gliomas remains unclear. CTP retains the advantage of a linear relationship between attenuation changes on CT and tissue concentration of contrast medium, unlike MRP [8, 20]. We therefore hypothesized that CTP should accurately provide quantitative information on only the microvasculature within the tumor, excluding extravasation due to permeability, when limited to patients with nonenhancing glioma. In the present study, we performed CTP on patients with nonenhancing glioma, and compared cerebral blood volume (CBV), cerebral blood flow (CBF), and mean transit time (MTT), as quantitative values provided from CTP, with postoperative histological diagnosis. The present study aims to determine whether CTP is useful for prediction of preoperative malignancy grading (WHO G2 or G3) in nonenhancing glioma on contrast-enhanced MRI.

Patients and methods

Patients

The study protocol was approved by the Ethics Committee of Iwate Medical University, Morioka, Japan. Consecutive patients admitted to the Department of Neurosurgery at Iwate Medical University between September 2006 and January 2010 and meeting the entry criteria were recruited to this study. Entry criteria for this study comprised: diagnosis of supratentorial glioma; tumor bulk not clearly enhanced on gadolinium-enhanced T1-weighted MRI (Gd-T1WI); tumor bulk sited in the supratentorial cerebrum; no past history relating to the brain, including surgical operation, irradiation, administration of anticancer agents or steroids, stroke, infection, or other disorders such as demyelinating disease; and provision of written informed consent to participate. Subjects comprised 17 patients (7 men, 10 women) with mean age of 47.8 years. Patient data including age, tumor site, operation method, postoperative histological diagnosis, and malignancy grade are summarized in Table 1.

Table 1 Patient summary

No.	Age (years)	Tumor site	Surgery	Histology	WHO grade
1	76	Temporal lobe	Biopsy	AA	3
2	58	Frontal lobe	Resection	AO	3
3	45	Frontal lobe	Resection	AO	3
4	34	Frontal lobe	Resection	AO	3
5	29	Frontal lobe	Resection	AO	3
6	21	Frontal lobe	Resection	AOA	3
7	78	Frontal lobe	Biopsy	DA	2
8	68	Frontal lobe	Biopsy	DA	2
9	68	Parietal lobe	Biopsy	DA	2
10	65	Frontal lobe	Resection	DA	2
11	58	Frontal lobe	Resection	DA	2
12	52	Frontal lobe	Resection	Oli	2
13	46	Temporal lobe	Resection	Oli	2
14	42	Frontal lobe	Resection	OA	2
15	30	Frontal lobe	Resection	OA	2
16	27	Frontal lobe	Resection	DA	2
17	16	Temporal lobe	Resection	OA	2

AA anaplastic astrocytoma, *AO* anaplastic oligodendroglioma, *AOA* anaplastic oligoastrocytoma, *DA* diffuse astrocytoma, *Oli* oligodendroglioma, *OA* oligoastrocytoma

Conventional MRI and CTP

Conventional MRI was performed for all subjects within 7 days before surgery. Spin-echo Gd-T1WI was performed approximately 2 min after intravenous injection of gadolinium (0.2 ml/kg, Magnevist; Bayer Schering Pharma, Berlin, Germany), using a 3.0-T whole-body scanner (GE Yokogawa Medical Systems, Tokyo, Japan) with a standard head coil. We confirmed that the tumor in each patient did not show clear enhancement with gadolinium on Gd-T1WI.

CTP was also performed within 7 days before surgery using a 16-row multidetector CT system (Aquillion 16; Toshiba Medical Systems, Tokyo, Japan), in accordance with the methods described by Sasaki et al. [23]. After performing noncontrast CT to determine the location of the tumor balk, a multislice scan targeting the tumor bulk was performed (80 kV_p; 40 mA; 1.5 s/rotation, 30 rotations field of view, 240 x 240 mm^2; four contiguous 8-mm-thick sections; total scan time, 45 s). Five seconds after intravenously injecting 40 ml (4 ml/s) nonionic iodine contrast medium (Iopamiron 300; Bayer Schering Pharma) using a power injector, dynamic scanning was started and tissue attenuation of contrast medium was monitored on a slice. Radiation doses for the scanning protocol were as follows: volume CT dose index, 150 mGy; dose–length product, 480 mGy cm; and effective dose, 1.34 mSv. Data were transferred to a commercial workstation (M900 Quadra; Ziosoft, Tokyo, Japan), and scaled color maps for CBF, CBV, and MTT were automatically created. All mathematical analyses were performed by the deconvolution method [19, 24], using CTP analysis software supplied with the workstation described above. Among the three types of deconvolution algorithms implemented in this software, we used the block-circulant singular value decomposition method. Regions of interest (ROI) for venous output and arterial input functions were manually placed at the superior sagittal sinus and a single branch of the insular segment of the middle cerebral artery on either the pathological or nonpathological side, or A2 segment of the anterior cerebral artery, respectively. ROI were also placed over the entire tumor bulk and apparently normal white matter (ANWM) on the nonpathological side, on color maps for each parameter. Size of the ROI for ANWM was established as 1.0 cm^2. In the measurement of absolute values, the vascular-pixel elimination (VPE) method was used to exclude pixels from large vessels at the cerebral surface, sulci, and cisterns [23, 25]. In the present study, we established the VPE threshold as 6.0 ml/100 g for CBV, since high-CBV areas suggesting large cortical vessels on color map disappeared satisfactorily at 6.0 ml/100 g when the threshold was varied between 5.0 and 8.0 ml/100 g using our analysis software. Large vascular pixels were thus defined as pixels with CBV values >6.0 ml/100 g and were automatically eliminated. Regional absolute values (*r*CBF, *r*CBV, and *r*MTT) were then calculated automatically for all ROI. The measurements described above were performed twice for each patient by two investigators (M.S. and K.K.) who were blinded to all clinical data, including individual patient information and histological diagnosis. Absolute values of all parameters for each patient were determined as the mean of four measured values, as determined twice by each investigator. The second test was performed 1 week after the first test, with a different randomized order of measurements from the first test. We also calculated normalized ratios (*n*CBF, *n*CBV, and *n*MTT) as the absolute value for the tumor divided by the absolute value for the ANWM for each parameter in all patients. All patients underwent surgery, with tumor resection for 13 patients and CT-guided stereotactic needle biopsy for 4 patients (Table 1). The region targeted in stereotactic biopsy was based on findings from the CBV color map. If the color map showed heterogeneous perfusion within the tumor, the targeted region corresponded to the region with the highest perfusion area for CBV. In cases with tumor resection, histological diagnosis was determined by observation at the lesion showing the most malignant histological features in all preparations. Postoperatively, histological diagnosis using specimens obtained from surgery was made by one of the investigators (A.K.) with no prior knowledge of CTP data.

Statistical analyses

All data were analyzed using PASW Statistics version 18 software (SPSS Japan, Tokyo, Japan). Inter- and intrarater reliabilities for all absolute values were evaluated according to classification of the intraclass correlation coefficient (ICC) [26]. For $ICC_{(1,1)}$ and $ICC_{(1,k)}$ as interrater reliability, agreement of all absolute values (CBF, CBV, and MTT) between first and second tests was analyzed for tumor and ANWM for each investigator, using one-factor analysis of variance (ANOVA). For $ICC_{(2,1)}$ and $ICC_{(2,k)}$ as intrarater reliability, agreement of all absolute values between the two investigators was analyzed for tumor and ANWM for each test, using two-factor ANOVA. Patients were assigned to one of two histological grading groups according to histological classification: WHO G2 or WHO G3. Frequency of biopsy was compared between G2 and G3 groups using Fisher's exact probability test. We compared absolute values from the tumor lesion for each parameter between G2 and G3 using the Mann–Whitney *U* test. Furthermore, the normalized ratio for each parameter was compared between these groups again using the Mann–Whitney *U* test. The accuracy of *r*CBF and *n*CBV in predicting a diagnosis of G3 was assessed using receiver

operating characteristic (ROC) curves. ROC curves were calculated in increments of 0.1. Absolute values and normalized ratios for CBV were compared between diffuse astrocytoma, G2 oligodendroglial tumor (OT), and G3 OT, using the Mann–Whitney U test. G2 OTs comprised oligodendroglioma or oligoastrocytoma, whereas G3 OTs comprised anaplastic oligodendroglioma or anaplastic oligoastrocytoma. Statistical significance was established at the $P < 0.05$ level in all analyses.

Results

Based on histological diagnosis after surgery, 6 patients were assigned to the G3 group and 11 patients were assigned to the G2 group (Table 1). Of these 17 patients, 4 patients underwent stereotactic biopsy. Frequency of biopsy did not differ significantly between G3 and G2 groups ($P = 0.25$).

Interrater reliability was classified as "almost perfect" for both tumor and ANWM for each investigator: $ICC_{(1,1)}$ and $ICC_{(1,k)}$ for M.S. were 0.943 and 0.971 for tumor, and 0.961 and 0.980 for ANWM, respectively, and those for K.K. were 0.966 and 0.983 for tumor, and 0.942 and 0.970 for ANWM, respectively. Intrarater reliability was also classified as "almost perfect" for both tumor and ANWM in each test: $ICC_{(2,1)}$ and $ICC_{(2,k)}$ in the first test were 0.987 and 0.993 for tumor, and 0.973 and 0.987 for ANWM, respectively, and those in the second test were 0.971 and 0.985 for tumor, and 0.973 and 0.986 for ANWM, respectively. Absolute values of tumor lesions for each parameter in G3 and G2 groups are summarized in Table 2. Absolute values for all parameters varied widely, with no significant differences in any parameters identified between G3 and G2 groups. Normalized ratios for each parameter are summarized in Table 3. Significant differences between G3 and G2 groups were identified for nCBF and nCBV, with no significant differences in nMTT.

The cutoff for accuracy was defined as the point lying closest to the upper-left corner of the ROC curve.

Table 2 Absolute values for each parameter

	rCBF (ml/100 g/min)	rCBV (ml/100 g)	rMTT (s)
G3 ($n = 6$)			
Range	10.8–27.0	1.9–3.2	6.8–10.8
Mean ± SD	18.3 ± 5.3	2.5 ± 0.5	8.5 ± 1.5
G2 ($n = 11$)			
Range	8.8–23.3	1.3–2.6	7.0–12.2
Mean ± SD	15.5 ± 4.2	2.1 ± 0.4	8.8 ± 1.5
P	0.27	0.25	0.76

SD standard deviation

Table 3 Normalized ratios for each parameter

	nCBF	nCBV	nMTT
G3 ($n = 6$)			
Range	1.34–3.00	1.54–2.39	0.76–1.06
Mean ± SD	2.10 ± 0.57	1.92 ± 0.37	0.90 ± 0.12
G2 ($n = 11$)			
Range	0.92–2.00	0.91–1.75	0.79–1.07
Mean ± SD	1.41 ± 0.38	1.26 ± 0.28	0.91 ± 0.09
P	0.01	0.004	0.76

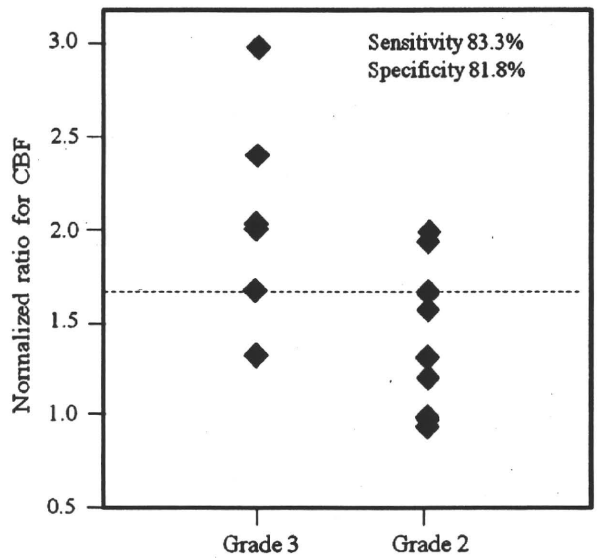

Fig. 1 Relationship between nCBF value and WHO grading. Using a cutoff of 1.7 (*dashed line*), nCBV was ≥1.7 for 5 (83.3%) of 6 patients with G3, compared with <1.7 for 9 (63.6%) of 11 patients with G2

Sensitivity and specificity in predicting a diagnosis of G3 were 83.3% and 81.8% for nCBF (cutoff 1.7), and 83.3% and 90.9% for nCBV (cutoff 1.6) (Figs. 1, 2). Accuracy for predicting a diagnosis of G3 was higher with nCBV than with nCBF.

A comparison of nCBV was made between G3 OT, G2 OT, and diffuse astrocytoma (Table 4). Significant differences in nCBV were identified between G3 and G2 OTs ($P = 0.009$), and between G3 OT and diffuse astrocytoma ($P = 0.02$), whereas no significant difference was seen between G2 OT and diffuse astrocytoma ($P = 0.36$).

Illustrative cases

We now describe the cases of two patients for whom CTP provided useful information for predicting tumor grading. Gd-T1WI for case 6 showed glioma with no clear enhancement in the right frontal lobe (Fig. 3a). Using the

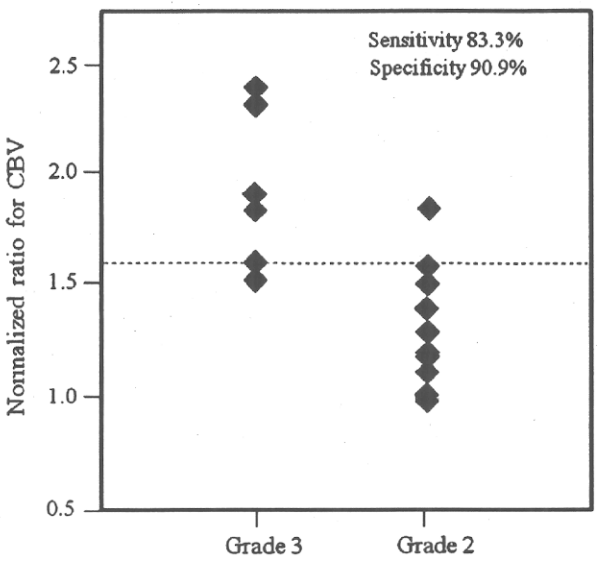

Fig. 2 Relationship between *n*CBV value and WHO grading. Using a cutoff point of 1.6 (*dashed line*), *n*CBV was ≥1.6 for 5 (83.3%) of 6 patients with G3 and <1.6 for 10 (90.9%) of 11 patients with G2

Table 4 Normalized ratio (mean ± SD) for CBV in G3 OT, G2 OT, and diffuse astrocytoma

	*n*CBV
G3 OT ($n = 5$)	1.99 ± 0.36
G2 OT ($n = 5$)	1.16 ± 0.24
Diffuse astrocytoma ($n = 6$)	1.35 ± 0.31

OT oligodendroglial tumors

VPE method, color mapping of CBV demonstrated large vessels of the cerebral surface to be successfully excluded (Fig. 3b). Color mapping of CBV depicted areas of hyperperfusion within the tumor. The *n*CBV for this case (*n*CBV = 2.3) was higher than the cutoff point. Tissue specimens obtained from gross total resection showed typical histological features of G3 anaplastic oligoastrocytoma.

Gd-T1WI for case 14 showed nonenhancing glioma of the right frontal lobe (Fig. 4a). The VPE method satisfactorily eliminated large vessels of the cerebral surface (Fig. 4b). On color mapping, areas of hyperperfusion seemed to be minor compared with those in case 6. The *n*CBV in this case (*n*CBV = 1.2) was lower than the cutoff point. After tumor resection, histological diagnosis was G2 oligoastrocytoma.

Discussion

Previous reports have documented that G3 gliomas make up 40–46% of nonenhancing gliomas on conventional MRI [3, 4]. Our finding of G3 tumors in 6 (35.2%) of 17 patients

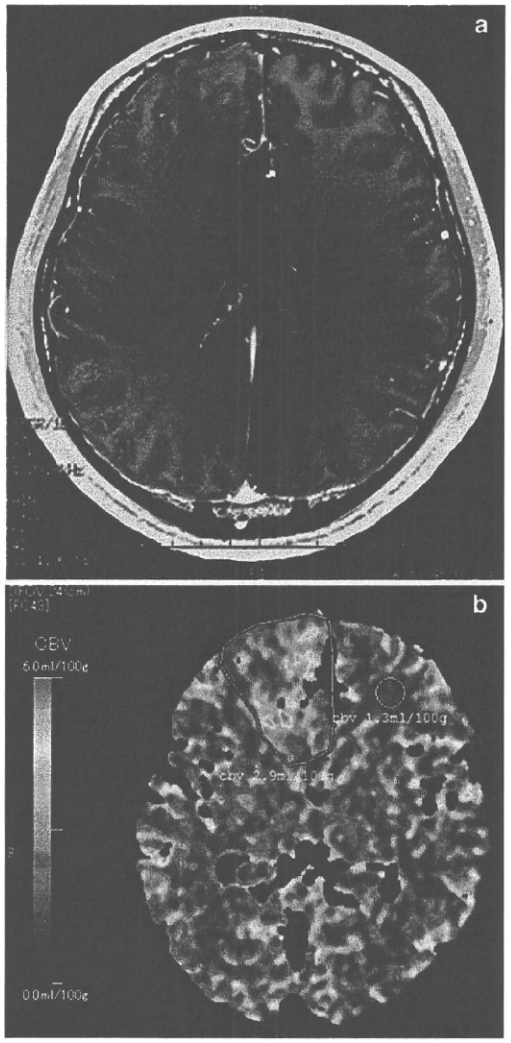

Fig. 3 Gd-T1WI (a) and color map of CBV (b) for case 6. *Circle* ROI covering the entire tumor bulk and ANWM localized on the nonpathological side

was close to this level. Thus, preoperative differentiation between G3 and G2 using MRI is often difficult. Biopsy or resection allowing histological diagnosis currently remain the basis for differentiation between G3 and G2 gliomas. However, neuroimaging can provide useful information on pathological diagnosis, particularly for patients who do not undergo biopsy or resection allowing histological diagnosis. Novel neuroimaging procedures other than routine MRI are thus desired. CTP and MRP provide reliable information on tumor vasculature, which can help to determine the extent of malignancy in glioma [8, 10, 22]. Although limitations of CTP include radiation dose and limited area of coverage compared with MRP, the linear relationship between attenuation changes on CT and tissue concentration of contrast medium and the lack of confounding sensitivity to flow artifacts allow CTP to

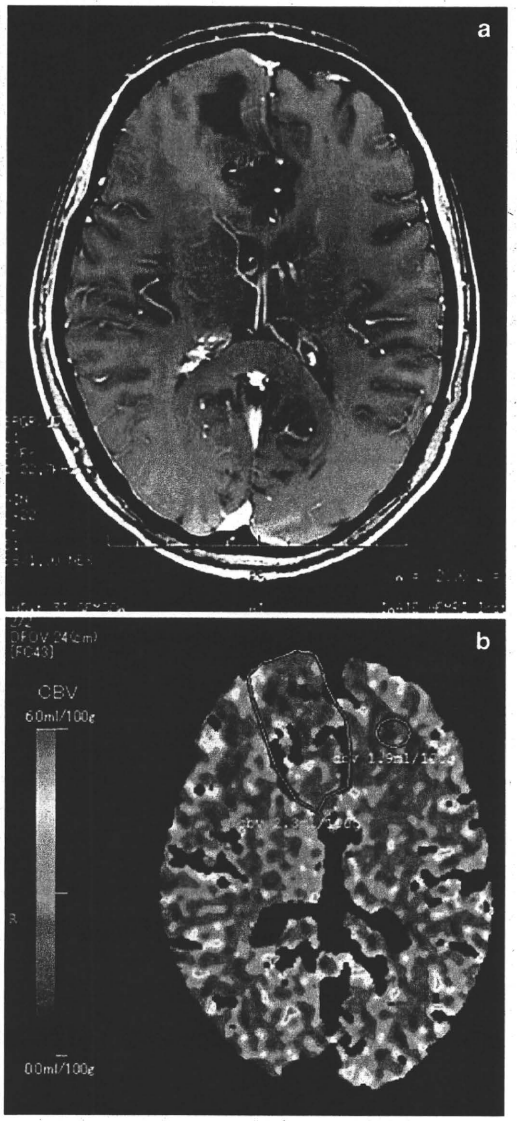

Fig. 4 Gd-T1WI (**a**) and color map of CBV (**b**) for case 14. *Circle* ROI covering the entire tumor bulk and ANWM localized on the nonpathological side

potentially offer a more accurate representation of tissue microvasculature than similar MRP studies [8, 20]. Furthermore, CTP offers advantages such as measurement of quantitative absolute values, greater availability, fast scanning time, high spatial resolution, low cost, and the ability to use this technique for patients who cannot undergo MRI due to the presence of metallic materials in the body [14, 22, 27].

CBF derived from CTP has been suggested to show a tendency toward overestimation, compared with that derived from PET [28]. Since overestimation of CBF in CTP was attributable to the presence of large vessels on the cerebral surface, as contrast materials act as a nondiffusible intravascular tracer in CTP unlike in PET, the VPE method has been proposed to eliminate flow in large vessels [25]. Accurate measurement of CBV contributes to accurate CBF and MTT, as these parameters are closely associated in the central volume principle as CBF = CBV/MTT [29]. We therefore used the VPE method in the present study. We think that optimal threshold differs according to the specific analysis software used for CTP. While VPE threshold was 8.0 ml/100 g in the report by Kudo et al. [25], we established a threshold of 6.0 ml/100 g, since high-CBV areas from cortical large vessels disappeared satisfactorily at this threshold for the analysis software used in our study. Another reason for using the VPE method is that OTs are commonly seen as superficially located tumors in the brain [30, 31]. Elimination of superficial large vessels at the cerebral surface, sulci, and cisterns thus seems warranted when CTP is performed for OTs.

In previous reports of CTP, *r*CBV values ranged from 2.3 to 8.87 ml/100 g for HGG and from 0.95 to 3.28 ml/100 g for LGG, differing significantly between HGG and LGG [13, 14, 20]. The present mean rCBV values in G3 and G2 (Table 2) agreed with previous findings. In addition, mean rCBV values in both G3 and G2 were less than half of 6.0 ml/100 g as VPE threshold. These findings suggest that the VPE method used in this study did not exclude tumor vessels along with other large vessels from CBV maps. While *r*CBV for G3 tended to be on the low side compared with previous reports, this could have resulted from the exclusion of patients with enhancing glioma as subjects in this study. Extravasation of contrast medium through the BBB in enhanced glioma may directly lead to increased CBV, due to the linear relationship between attenuation changes on CT and tissue concentration of contrast medium. Jain et al. [20] documented that *r*CBF and *r*CBV in nonenhancing G3 glioma do not differ significantly from those in nonenhancing G2 glioma, although sample size in that report was small. The present study with more subjects suggested that even nonenhancing G3 glioma retains more vascular density than G2, although the difference in *r*CBV between the two groups was minor (Table 2). However, this result might have been influenced by the disproportionate number of OTs in the G2 (42%) and G3 (83%) groups. If vascular density is significantly higher in G3 OT than in anaplastic astrocytoma, the large number of G3 OTs may have result in a high mean CBV for the G3 group in this study. This issue represents a definite limitation to the present study.

Concentration of contrast medium within the tumor might be subtly influenced by individual parameters such as body size and cardiac output volume, and differences in analytical software among institutes. We must emphasize the importance of estimation using normalized ratios, as