Discrimination between low-grade oligodendrogliomas and diffuse astrocytoma with the aid of $^{11}$C-methionine positron emission tomography

Clinical article

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Object. The diagnostic usefulness of $^{11}$C-methionine PET scans in gliomas is still controversial. The authors investigated the clinical significance of $^{11}$C-methionine PET findings in preoperative diagnosis of histological type and grade.

Methods. The tissue uptake of $^{11}$C-methionine was assessed using PET in 70 patients with histologically confirmed intracerebral gliomas. The ratio of maximum standard uptake values in tumor areas to the mean standard uptake values in the contralateral normal brain tissue (tumor/normal tissue [T/N] ratio) was calculated and correlated with tumor type, histological grade, contrast enhancement on MR imaging, Ki 67 labeling index, and 1p/19q status.

Results. The T/N ratio was significantly increased as tumor grade advanced in astrocytic tumors (WHO Grade II vs Grade III, $p = 0.0011$; Grade III vs Grade IV, $p = 0.0007$). Among Grade II gliomas, the mean T/N ratio was significantly higher in oligodendrogial tumors than in diffuse astrocytomas (DAs) ($p < 0.0001$). All T/N ratios for oligodendroglial tumors were $\geq 1.46$, and those for DA were consistently $< 1.46$, with the exception of 2 cases of gemistocytic astrocytoma. The Ki 67 labeling index significantly correlated with T/N ratio in astrocytic tumors, but not in oligodendrogliomas. Oligodendrogial tumors without 1p/19q deletion had a significantly higher T/N ratio than those with the codeletion. In combination with Gd-enhanced MR imaging, 67% of nonenhanced tumors with a T/N ratio of $\geq 1.46$ were proved to be Grade II oligodendrogliomas.

Conclusions. These results clearly show that $^{11}$C-methionine PET T/N ratios in Grade II oligodendrogliomas were higher than those in DAs independently of their proliferative activity. This information contributes to preoperative differential diagnoses of histological type, especially in suspected low-grade gliomas.

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**Key words** • positron emission tomography • methionine • glioma • oligodendroglial tumor • 1p/19q deletion

Tumors of glial origin, such as astrocytomas or oligodendrogliomas, comprise the majority of human brain tumors. The imaging modalities that are conventionally used, such as contrast-enhanced CT scanning and MR imaging, provide excellent information on anatomical localization and very little information about biological features of gliomas. Indeed, these structural imaging modalities are usually insufficient in the prediction of histological type and grade. Some tumors suspected to be WHO Grade II gliomas are histologically diagnosed as DA, whereas others with identical imaging findings are identified as oligodendroglioma. The structural image is thus expected to be supplemented by biological information contributing to the histological diagnosis.

Strategies for imaging the biological features of tissues by mapping the uptake or usage of nutrients such as glucose, amino acids, and nucleotides are useful in noninvasive studies of normal functions and for detection of pathological lesions. Positron emission tomography performed using L-[methyl-$^{11}$C]methionine is the most popular amino acid imaging modality for tumors, although its...
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use is restricted to PET centers with an in-house cyclotron facility.\textsuperscript{15,22} Positron emission tomography performed using $^{11}$C-methionine has been used in attempts to analyze intracerebral gliomas. It has been established that this method is useful in detecting low-grade glioma when other imaging modalities yield inconclusive results. The $^{11}$C-methionine PET modality can potentially distinguish high-grade from low-grade glioma,\textsuperscript{5,17,24,28} whereas some controversy remains about its ability to discriminate between Grade II glioma and Grade III tumor.\textsuperscript{5,16} Furthermore, it is generally believed that histological tumor types cannot be accurately predicted by $^{11}$C-methionine PET.\textsuperscript{28} Many other tumor types, including metastatic brain tumors, malignant lymphoma, and meningiomas, also have high methionine uptake, which cannot be distinguished from that in gliomas.\textsuperscript{5,13,25}

In spite of these limitations, such a biological imaging study may be informative for preoperative supplementary diagnosis of histological type and grade because it can evaluate whole tumor areas. Recently, since low-grade oligodendroglioma became known as relatively chemosensitive and usually had a more favorable outcome than DA,\textsuperscript{12,22} preoperative information on whether an intracerebral tumor has oligodendroglial components would be invaluable for clinical decision making. To evaluate the contribution of $^{11}$C-methionine PET to the preoperative discrimination of tumor histological type and grade, a possible correlation of the quantitative results of $^{11}$C-methionine PET with histological diagnosis, Ki 67 LI, and lp/19q status was analyzed in patients with glioma.

**Methods**

**Patient Population**

A retrospective review of medical records was performed in patients who had undergone $^{11}$C-methionine PET as a part of diagnostic tumor investigation at the National Institute for Radiological Science or at Chiba Medical Center for Prolonged Traumatic Brain Dysfunction between 2002 and 2009. We obtained the institutional review board’s approval for the study protocol, and each of the patients provided written informed consent. A total of 70 patients were included in this retrospective study (Table I). Most patients underwent a maximum resection of the tumor, including the areas of highest $^{11}$C-methionine uptake. When such a maximum resection was not possible, sampling of the highest uptake areas was done using the navigation system or a stereotactic biopsy procedure.

All tumor materials were classified and graded histologically according to the WHO classifications: 3 patients with pilocytic astrocytoma, 3 with ganglioglioma, 12 with diffuse or gemistocytic astrocytoma, 20 with oligodendroglial tumors (16 with oligodendroglioma and 4 with oligoastrocytoma), 11 with anaplastic astrocytoma, 8 with anaplastic oligodendrogiomas, and 13 with glioblastoma. The group comprised 49 men and 21 women with a mean age of 48 years (range 23–74 years). Twenty-four patients (34%) underwent total resection, 15 (22%) underwent subtotal resection, 29 (41%) underwent partial resection, and 2 (3%) underwent stereotactic biopsy sampling. All patients underwent surgical procedures within 2 weeks after PET.

**The $^{11}$C-Methionine PET Scans**

The $^{11}$C-methionine PET scanning was performed using high-resolution full-ring scanners (Biograph, Siemens/CTI; or Discovery ST-E, GE Healthcare), with spatial resolutions of 4.5 and 4.8 mm, respectively. Patients fasted for $\geq$ 4 hours before PET scanning, and PET images were acquired with the patient in a resting state. Static scanning was performed for 6 minutes in 3D acquisition and reconstructed using a 3D ordered-subset expectation maximization algorithm. On the Discovery ST-E scanner, CT-based attenuation-corrected PET images were used. A methionine dose of 370–720 MBq was injected intravenously within 1 minute, with the scan starting 20 minutes after methionine injection. Summation images covering 20–40 minutes after injection were used for analyses. Accurate coregistration of CT and PET images was performed using commercially available software (Advantage Workstation, GE Healthcare).

In each tumor, we looked at a circular ROI with a diameter of 10 mm in the hot spot of each lesion. If increased accumulation was absent, an ROI was selected in consultation with the fused CT and MR images. Regional uptake of tracer was expressed as an SUV, which is the activity concentration in the ROI at a fixed time point, divided by the injected dose, and normalized to the measured weight of the patient. We also took the ROI of 5 points in the contralateral normal brain cortex and calculated the average. Tumor uptake was expressed as the ratio of the maximum SUV for the tumor to the mean SUV for contralateral normal brain cortex (the T/N ratio).

**Magnetic Resonance Imaging**

The MR imaging was performed on a 1.5-T system (Signa, GE Medical Systems). Axial T1-weighted images were obtained after administration of 0.2 ml/kg of gadopentetate by using the following parameters: 6-mm thickness, 1.5-mm gap, TR 300 msec, TE 23 msec, number of excitations 1).

**Chromosome 1p and 19q Deletion Analysis and Immunohistochemistry**

Chromosome lp and 19q deletion analyses were done using standard FISH analysis of fixed cytogenetic preparations from fresh tumor tissues. The FISH probes for lp were the target region of lp36, with a control region of lp25, and those for 19q were a control region of 19q13, with the target region of 19q13. The total number of signals was counted, and the ratio of 1p:1q or 19q:19p of $< 0.75$ was diagnosed as loss. Immunohistochemical examination of paraffin-embedded samples with MIB-1 monoclonal antibody against the Ki 67 antigen (Immunotech) was performed using commercially available reagents and according to the manufacturer’s recommendations. The Ki 67 antigen was retrieved by autoclaving. The samples were incubated with the antibody overnight in the same buffer, followed by incubation with the biotinylated secondary antibody.
The bound antibodies were visualized by the avidin biotinylated peroxidase complex method and diaminobenzidine tetrachloride. For quantitative MIB-1 evaluations, the positive cells in fields viewed at a magnification of 200 (minimum of 1000 nuclei) were counted, and the LI was expressed as the percentage of the labeled tumor cells. This scoring was done in a blinded fashion, with no knowledge of the patient outcome.

Statistical Analysis

Differences in SUV T/N ratios among various grades of gliomas were tested by 1-way ANOVA and the Fisher protected least significant difference method. The unpaired t-test was used to test the correlation between 2 groups. Receiver operating characteristics curve analysis was used to determine the optimal cutoff values for the differential diagnosis of DA and oligodendrogliomas. The correlation between Ki 67 LI and T/N ratio was analyzed using the nonparametric Spearman rank test. Statistical analyses were performed using Stat-View and SAS software (SAS Institute, Inc.).

Results

The T/N Ratio and Tumor Grade

The mean ± SD for the SUV T/N ratio in all examinations was 2.19 ± 1.27, and distributions of T/N ratios in each tumor type and grade are presented in Table 1. The T/N ratio tended to increase as tumor grade advanced, but no significant correlation was identified. A wide variation in T/N ratios (from 0.63 to 4.54) was seen in Grade II lesions, including DA and oligodendrogliomas. For astrocytic tumors, the T/N ratio increased significantly in proportion to the advance of tumor grade (p = 0.0011 between Grades II and III; p = 0.0007 between Grades III and IV) (Fig. 1).

In contrast, there was no significant difference in T/N ratio between Grade II oligodendrogliomas and Grade III anaplastic oligodendrogliomas (p = 0.101) (Fig. 2).

Comparison of T/N Ratios Between Oligodendrogliomas and Astrocytic Tumors

We next focused on WHO Grade II tumors. Illustrative images from patients with DAs and Grade II oligodendrogliomas are shown (Figs. 3 and 4). The mean T/N ratio was 1.06 ± 0.26 for nongemistocytic DA and 2.38 ± 0.74 for Grade II oligodendrogliomas (Table 1), and a significant difference was seen between these histological types (p < 0.0001). The receiver operating characteristics curve analysis showed that a cutoff T/N ratio of 1.46 provided one of the best sensitivity and specificity levels for differential diagnosis in the present cohort. All T/N ratios for Grade II oligodendrogliomas were ≥ 1.46 (range 1.46–4.54), and the ratios for nongemistocytic DA were < 1.46 (range 0.63–1.37). The 2 patients with gemistocytic astrocytoma had specifically high T/N ratios among DAs (minimum and maximum T/N ratio of 1.70 and 2.70, respectively; Table 1). When gemistocytic astrocytomas were excluded from the DA group, no overlap of range in the distributions of T/N ratio was observed between these 2 histopathological categories on 11C-methionine PET scans (Fig. 5).

The Ki 67 LI and the T/N Ratio

The Ki 67 LI was significantly correlated with the...
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T/N ratio for all gliomas (p = 0.007, Spearman rank test; data not shown). When astrocytic tumors and oligodendrogliomas were analyzed separately, the Ki 67 LI showed a significant correlation with the T/N ratio in astrocytic tumors, but not in oligodendrogliomas (p = 0.0129 and p = 0.18, respectively) (Fig. 6). This result indicates that the relatively high T/N ratio in Grade II oligodendrogliomas does not reflect proliferative activity.

The 1p/19q Codeletion and the T/N Ratio in Oligodendrogial Tumors

The 1p/19q deletion was observed in 17 (71%) of 24 patients with oligodendrogial tumors analyzed using FISH; in 13 (72%) of 18 patients with Grade II oligodendrogial tumors; and in 4 (67%) of 6 patients with Grade III tumors. The mean T/N ratio was 2.35 ± 0.78 for the 1p/19q deleted tumors and 3.25 ± 0.79 for the nondeleted oligodendrogial tumors (Fig. 7). Oligodendrogial tumors without 1p/19q deletion had a significantly higher T/N ratio than those with codeletion (p = 0.0173). When the analysis was restricted to Grade II tumors, the same result was obtained, with a greater statistical significance (p = 0.0018).

Preoperative Histological Prediction in Combination With Contrast-Enhanced MR Imaging

Contrast enhancement on MR imaging was observed in 100% of glioblastoma and 63% of anaplastic gliomas, whereas it was observed in only 20% of low-grade oligodendrogial tumors and 0% of DAs (Table 2). Because most of the malignant gliomas had contrast enhancement and a large part of the low-grade gliomas were not...
enhanced, a T/N ratio of ≥ 1.46 in nonenhanced tumors would suggest that the tumor is more likely to be a low-grade oligodendroglial tumor than a DA.

The T/N Ratio and Patient Survival

We next examined the correlation between T/N ratio and patient prognosis in low-grade gliomas. In oligodendroglial tumors, although the T/N ratio differed significantly, prognosis and chemosensitivity were favorable in all patients, with a 5-year survival rate of 100% regardless of T/N ratio. In nongemistocytic DAs, the T/N ratio fell into the narrow range of 0.63–1.37, and no correlation was seen between T/N ratio and survival periods. Two patients with a maximum T/N ratio of 1.36 and 1.37 remained alive for 11 years and 5 years, respectively. One of the patients with gemistocytic astrocytoma experienced tumor recurrences, but lived for 4 years after salvage surgery. The other patient has been alive without tumor recurrence for 3 years.

Discussion

Our study clearly showed that the mean SUV T/N ratio in low-grade oligodendroglial tumors was significantly higher than that found in nongemistocytic DAs by using $^{11}$C-methionine PET. Although categorized in the same histological grade, the T/N ratio was shown to differ by histological type. This result suggests its possible contribution in discriminating between low-grade oligodendroglial tumors and DAs when combined with other structural imaging modalities, such as contrast-enhanced MR imaging, to exclude malignant gliomas also present.
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Fig. 5. Scatter diagram demonstrating differences in methionine SUVs of the T/N ratio depending on histological type as either Grade II astrocytoma or oligodendroglioma. The mean SUV T/N ratio was significantly higher in Grade II oligodendrogliomas than in Grade II astrocytoma ($2.38 \pm 0.74$ and $1.06 \pm 0.26$, respectively; $p < 0.0001$, unpaired t-test). The horizontal line shows a cutoff threshold of 1.46 for the T/N ratio. All oligodendrogliomas exist above this line, whereas all non-gemistocytic DAs are under this threshold.

Fig. 6. Correlation of the methionine SUVs for T/N ratios with Ki 67 LI. Left: The T/N ratio shows a positive correlation with Ki 67 in 20 astrocytic tumors ($p = 0.0129$). Right: The T/N ratio shows no correlation with Ki 67 LI in 19 oligodendrogliomas ($p = 0.18$).

Fig. 7. Scatter diagram demonstrating differences in methionine SUVs of T/N ratio depending on 1p/19q status in oligodendrogliomas. The mean SUV T/N ratio was significantly higher in oligodendrogliomas without 1p/19q deletion than in those with 1p/19q deletion ($3.25 \pm 0.79$ and $2.35 \pm 0.78$, respectively; $p = 0.0173$, unpaired t-test).

Loss of chromosomes 1p and 19q is found in 60%–90% of oligodendroglial tumors. The presence of this deletion has been found to correlate with longer survival and chemosensitivity, especially in Grade III tumors. Although it has been shown that 1p/19q deletion is exclusively associated with p53 overexpression or unmethylated methylguanine methyltransferase, few biological characteristics are known to be linked to this cytogenetic abnormality. In the present study, we report for the first time a significantly higher T/N ratio in $^{11}$C-methionine PET for the oligodendrogliomas without 1p/19q deletion than in those with the codeletion. The higher T/N ratio in 1p/19q nondeleted oligodendrogliomas would reflect some biological effects of unknown genetic changes other than 1p/19q deletion. However, because the sample size was small in the present analysis, the result should be confirmed in a future study involving more patients with 1p/19q status information.

The main mechanism of amino acid uptake into the
cytoplasm of the tumor involves a sodium-dependent transport system in the cell membrane. This transport system is overexpressed in tumor cells, and the expression level seems to be correlated to tumor cell growth rates. Various factors can influence activity of the transport system, including pH, hormones, growth factors, amino acid availability, and cellular proliferation rate. Our current results show that the Ki 67 LI significantly correlated with the T/N ratio in astrocytic tumors, but not in oligodendrogliomas, and that there was no significant difference in T/N ratio between Grade II and Grade III oligodendrogliomas. These results collectively indicate that the high T/N ratio in Grade II oligodendroglioma does not simply reflect proliferative activity. The mechanism of high methionine uptake in oligodendrogliomas would be mainly attributed to higher cell density, which would make the transport system work more densely in the tumor tissue. Other researchers have suggested higher microvessel density as a cause of higher methionine uptake in oligodendrogliomas. Angiogenic processes in this tumor would increase the carrier-mediated large amino acid transport.

In DAs, some tumors progress to glioblastoma, whereas others persist in a dormant state for many years. This biological heterogeneity makes an accurate prognosis and the selection of appropriate therapeutic strategies rather difficult. Although some degree of controversy remains, many previous reports have shown that a higher T/N ratio of methionine uptake is associated with shorter survival in patients with cerebral glioma. The significant correlation between histological grade in astrocytic tumors and methionine T/N ratio in our study is consistent with the reported positive results. However, when analysis was limited to DAs, almost all T/N ratios fell into a narrow range between 0.63 and 1.37, and no correlation was observed between T/N ratio and duration of survival. The 2 patients with gemistocytic astrocytoma were still alive in spite of the relatively high methionine uptake. In contrast, some authors have described high methionine uptake as an independent prognostic factor in Grade II astrocytoma. Our series was not large enough to allow each grade to be considered separately with sufficient statistical power. Further analyses including more patients are needed to clarify the prognostic value of 11C-methionine PET studies in patients with DA.

The limitations of this study are the small sample size and the retrospective nature of the study design. The clinically important information about 11C-methionine PET obtained in this study needs to be validated in a larger prospective trial with an independent cohort of patients.

### References


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