Diagnostic Performance of [^{11}C]Methionine Positron Emission Tomography in Newly Diagnosed and Untreated Glioma Based on the Revised World Health Organization 2016 Classification

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■ BACKGROUND: **The relationship between uptake of amino acid tracer with positron emission tomography (PET) and glioma subtypes/gene status is still unclear.**

■ OBJECTIVE: **To assess the relationship between uptake of [^{11}C]methionine using PET and pathology, *IDH* (isocitrate dehydrogenase) mutation, 1p/19q codeletion, and *TERT* (telomerase reverse transcriptase) promoter status in gliomas.**

■ METHODS: **The participants were 68 patients with newly diagnosed and untreated glioma who underwent surgical excision and preoperative [^{11}C]methionine PET examination at Osaka City University Hospital between July 2011 and March 2018. Clinical and imaging studies were reviewed retrospectively based on the medical records at our institution.**

■ RESULTS: **The mean lesion/contralateral normal brain tissue (L/N) ratio of diffuse astrocytomas was significantly lower than that of anaplastic astrocytomas ($P = 0.00155$), glioblastoma ($P < 0.001$), and oligodendrogliomas ($P = 0.0157$). The mean L/N ratio of *IDH* mutant gliomas was significantly lower than that of *IDH* wild-type gliomas (median 1.75 vs. 2.61; $P = 0.00162$). A mean L/N ratio of 2.05 provided the best sensitivity and specificity for distinguishing between *IDH* mutant and *IDH* wild-type gliomas (69.2% and 76.2%, respectively). The mean L/N ratio of *TERT* promoter mutant gliomas was significantly higher than that of *TERT* promoter wild-type gliomas ($P = 0.0147$). Multiple regression analysis showed that pathologic diagnosis was the only influential factor on L/N ratio.**

■ CONCLUSIONS: **Distinguishing glioma subtypes based on the revised 2016 World Health Organization classification of the central nervous system tumors on the basis of [^{11}C]methionine PET alone seems to be difficult. However, [^{11}C]methionine PET might be useful for predicting the *IDH* mutation status in newly diagnosed and untreated gliomas noninvasively before tumor resection.**

INTRODUCTION

Gliomas are most the common tumors in Japan, accounting for 25.6% of primary brain tumors in the country.[1] In the United States, gliomas are the second most common form of primary brain tumors, with an annual incidence rate of approximately 6 cases per 100,000 people.

Key words
- [^{11}C]methionine
- Glioma
- *IDH*
- L/N ratio
- PET
- *TERT* promoter

Abbreviations and Acronyms
AUC: Area under the curve
IDH: Isocitrate dehydrogenase
IQR: Interquartile range
L/N: Lesion/contralateral normal brain tissue
MRI: Magnetic resonance imaging
PET: Positron emission tomography
ROC: Receiver operating characteristic
ROI: Region of interest
TERT: Telomerase reverse transcriptase
WHO: World Health Organization

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Nearly 50% of primary malignant brain tumors are glioblastomas, and approximately 17% are other astrocytomas.[2] The revised 2016 World Health Organization (WHO) classification of tumors of the central nervous system requires molecular classification such as isocitrate dehydrogenase (*IDH*) mutation and 1p/19q codeletion for a diagnosis of glioma.[3]

Magnetic resonance imaging (MRI) is the gold standard for diagnosing glioma; however, it can be difficult to distinguish glioma from other nonneoplastic lesion such as radiation necrosis. [^{11}C]Methionine positron emission tomography (PET) has been widely used in patients with glioma for detecting the tumor, deciding biopsy target location,[4-6] predicting the grade[7-11] and prognosis,[8,10,12-15] evaluating therapeutic response,[10,16] and distinguishing tumor recurrence from radiation necrosis.[17-19] However, the relationship between the uptake of amino acid tracer with PET and glioma subtypes/gene status is still unclear. The aim of this study was to evaluate the association between [^{11}C] methionine uptakes and disease/gene status in newly diagnosed and untreated gliomas.

METHODS

Patients

A total of 68 patients (42 males, 26 females; mean age, 51.8 years; age range, 7–82 years) with newly diagnosed and untreated gliomas underwent surgical resection at Osaka City University Hospital between July 2011 and March 2018. [^{11}C]Methionine PET was performed within 1 month before the tumor resection in patients with glioblastoma (median, 12.7 days; interquartile range [IQR], 7–15.5 days) and within 6 months in patients with lower-grade glioma (median, 41.8 days; IQR, 10–59.8 days). This study was approved by the institutional review boards of the Graduate School of Medicine of Osaka City University (approval numbers 2047 and 2020-115) and Osaka National Hospital (approval number 0713). Genetic analyses were performed with patients' written consent.

[^{11}C]Methionine PET

PET was performed using an Eminence B PET scanner (Shimadzu, Kyoto, Japan) (spatial resolution, 4.5 mm [full width at half maximum]; slice thickness, 5.6 mm) or a Biograph-16 PET scanner (Siemens, Bonn, Germany; spatial resolution, 4.6 mm [full width at half maximum]; slice thickness, 5.1 mm). Scanning was performed parallel to the orbitomeatal line of the patients. During a period of fasting, 6 MBq/kg of [^{11}C]methionine was injected intravenously over 30 seconds. After a transmission scan was obtained, a 10-minute static scan was begun 20 minutes after injection. PET data were analyzed using the same region of interest (ROI) settings as previously reported.[17] Irregular ROIs were manually placed in the coregistered MRI for each lesion and the contralateral cerebral cortex. ROIs were transferred to the corresponding PET image to calculate the uptake of [^{11}C] methionine. Activity counts were normalized relative to the injected dose per kilogram of patient body weight (standardized uptake value). The mean and maximum standardized uptake values were calculated by semiquantitative analysis of [^{11}C] methionine uptake by each lesion. The mean and maximum lesion/contralateral normal brain tissue (L/N) ratios were determined by dividing the tumor standardized uptake value by the mean standardized uptake value of the normal contralateral region of the brain, as previously reported.[17,20]

Gene Analysis

Genomic tumor DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, California, USA) or NucleoSpin Tissue (Machery-Nagel, Duren, Germany), and hot spots mutations of *IDH*1/2 (codon 132 of *IDH*1 and codon 172 of *IDH*2) and telomerase reverse transcriptase (*TERT*) promoter (termed C228 and C250) by Sanger sequencing with a 3130xLGenetic Analyzer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and Big-Dye Terminator V1.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA), as previously reported.[21,22] The copy number status of 1p19q was determined by multiplex ligation-dependent probe amplification (Oligodendroglioma1p-19qprobemix and EK1 reagent kit [MRC-Holland, Amsterdam, Netherlands]).

Statistical Analysis

All data were evaluated using EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphic user interface for R (R Foundation for Statistical Computing, Vienna, Austria). It is a modified version of R commander designed to add statistical functions frequently used in biostatistics.[23] To compare the scores of the mean and maximum L/N ratios of each group, we performed statistical analysis using a Kolmogorov-Smirnov test, Kruskal-Wallis test, and Mann-Whitney U test. Receiver operating characteristic (ROC) curves were analyzed to determine the best cutoff value for differentiating gliomas with *IDH* mutant from those with *IDH* wild-type and those with *TERT* promoter mutant from those with *TERT* promoter wild-type. Multiple regression analysis was used to evaluate influential factors except for the patient with anaplastic oligodendroglioma ($n = 1$) on L/N ratio. Statistical significance was defined at the level of $P < 0.05$.

RESULTS

The 68 tumors were classified according to the revised 2016 WHO classification of tumors of the central nervous system (**Tables 1 and 2**). Eleven patients were classified into *IDH* mutant diffuse astrocytoma, 9 patients were classified with *IDH* wild-type diffuse astrocytoma, 8 with *IDH* mutant and 1p/19q codeleted oligodendroglioma, 2 with *IDH* mutant anaplastic astrocytoma, 9 with *IDH* wild-type anaplastic astrocytoma, 1 patient with *IDH* mutant and 1p/19q codeleted anaplastic oligodendroglioma, 4 patients with *IDH* mutant glioblastoma, and 24 with *IDH* wild-type glioblastoma. Patients were further subdivided into 4 types based on *IDH* and *TERT* promoter mutation status (**Table 1**). Group A (*IDH* mutant/*TERT* promoter mutant) comprised 9 patients, with pathologic diagnoses of *IDH* mutant and 1p/19q-codeleted oligodendroglioma ($n = 7$), anaplastic oligodendroglioma ($n = 1$), and glioblastoma ($n = 1$). Group B (*IDH* mutant/*TERT* promoter wild-type) comprised 17 patients, with pathologic diagnoses of diffuse astrocytoma ($n = 11$), glioblastoma ($n = 3$), anaplastic astrocytoma ($n = 2$) and *IDH* mutant and 1p/19q

Table 1. Patients' Characteristics and Pathology Based on the Revised 2016 World Health Organization Classification

Characteristics	Value	%
Age (years), median (range)	55.5 (7–82)	
Sex		
Male	42	61.8
Female	26	38.2
Contrast enhancement on magnetic resonance imaging		
Yes	46	67.6
No	22	32.4
Histology		
Diffuse astrocytoma	20	29.4
IDH mutant	11	16.2
IDH wild-type	9	13.2
Oligodendroglioma, *IDH* mutant and 1p19q-codeleted	8	11.8
Anaplastic astrocytoma	11	16.2
IDH mutant	2	2.9
IDH wild-type	9	13.2
Anaplastic oligodendroglioma, *IDH* mutant and 1p19q-codeleted	1	1.5
Glioblastoma	28	41.2
IDH mutant	3	4.4
IDH wild-type	25	36.8
IDH/TERT promoter status		
Group A: *IDH* mutant/*TERT* promoter mutant	9	13.2
Group B: *IDH* mutant/*TERT* promoter wild-type	17	25.0
Group C: *IDH* wild-type/*TERT* promoter wild-type	23	33.8
Group D: *IDH* wild-type/*TERT* promoter mutant	19	27.9

IDH, isocitrate dehydrogenase; *TERT*, telomerase reverse transcriptase.

Table 2. Subgroups Based on *IDH/TERT* Promoter Status

	TERT Promoter Mutant (n = 28)	*TERT* Promoter Wild-Type (n = 40)
IDH mutant (n = 26)	Oligodendroglioma 7 (77.8%) Anaplastic oligodendroglioma 1 (11.1%) GBM 1 (11.1%)	Diffuse astrocytoma 11 (64.7%) GBM 3 (17.6%) Anaplastic astrocytoma 2 (11.8%) Oligodendroglioma 1 (5.9%)
IDH wild-type (n = 42)	GBM 14 (73.7%) Anaplastic astrocytoma 3 (15.8%) Diffuse astrocytoma 2 (10.5%)	GBM 10 (43.5%) Diffuse astrocytoma 7 (30.4%) Anaplastic astrocytoma 6 (26.1%)

TERT, telomerase reverse transcriptase; *IDH*, isocitrate dehydrogenase; GBM, glioblastoma.

codeleted oligodendroglioma (n = 1). Group C (IDH wild-type/TERT promoter mutant) comprised 23 patients, with pathologic diagnoses of glioblastoma (n = 10), diffuse astrocytoma (n = 7), and anaplastic astrocytoma (n = 6). Group D (IDH wild-type/TERT promoter wild-type) comprised 19 patients, with pathologic diagnoses of glioblastoma (n = 14), anaplastic astrocytoma (n = 3), and diffuse astrocytoma (n = 2) (Table 2).

Table 3 lists the mean and the maximum L/N ratio of gliomas classified according to WHO grade, disease, IDH status, and TERT promoter status.

Correlation Between L/N Ratio and Pathologic Diagnosis

The medians of the mean L/N ratios of WHO grade II, III, and IV gliomas were 1.60 (IQR, 1.00–2.06), 2.26 (IQR, 1.80–4.20), and 3.02 (IQR, 2.50–3.42), respectively ($P < 0.001$). Significant differences were found between grade II and III gliomas ($P = 0.0042$) and between grade II and IV gliomas ($P < 0.001$), but not between grade III and IV gliomas (Figure 1A). The mean L/N ratio of high-grade gliomas (median, 2.93; IQR, 2.28–3.49) was significantly higher than that of low-grade gliomas (median, 1.60; IQR, 1.00–2.06; $P < 0.001$). The mean L/N ratio of glioblastomas (median, 3.02; IQR, 2.50–3.42) was significantly higher than that of diffuse astrocytomas (median, 1.20; IQR, 0.75–1.86; $P < 0.001$) and oligodendrogliomas (median, 2.03; IQR, 1.82–2.35; $P < 0.001$). The mean L/N ratio of diffuse astrocytomas was significantly lower than that of anaplastic astrocytomas (median, 2.06; IQR, 1.67–3.32; $P = 0.00155$) and oligodendrogliomas ($P = 0.0157$) (Figure 1B).

Correlation Between L/N Ratio and Pathologic Diagnosis with IDH Status

Among grade II gliomas, oligodendrogliomas showed a significantly higher mean L/N ratio compared with IDH mutant diffuse astrocytomas (median, 1.08; IQR, 0.63–1.65; $P < 0.001$). There was no statistically significant difference between oligodendrogliomas and IDH wild-type diffuse astrocytomas (median, 1.58; IQR, 1.02–2.14) (Figure 2A). Among grade III gliomas, there was no significant difference among anaplastic oligodendrogliomas, IDH mutant anaplastic astrocytomas, and IDH wild-type anaplastic astrocytomas (Figure 2B); there was also no significant difference between IDH mutant and IDH wild-type glioblastomas (Figure 2C).

Correlation Between L/N Ratio and *IDH/TERT* Promoter Status

The medians of the mean L/N ratios of groups A, B, C, and D were 2.08 (IQR, 1.89–2.67), 1.68 (IQR, 0.96–1.80), 2.49 (IQR, 1.80–3.21), and 2.92 (IQR, 2.26–3.14), respectively ($P = 0.00223$) (Table 3). Statistically significant differences in the mean L/N ratio were found between groups A and B ($P = 0.0336$), B and C ($P = 0.009$), and B and D ($P = 0.000162$) (Figure 3A). The medians of the maximum L/N ratios of group A, B, C, and D were 3.27 (IQR, 2.55–3.79), 2.49 (IQR, 1.60–3.08), 4.38 (IQR, 2.73–5.20), and 4.56 (IQR, 3.55–4.90), respectively. Statistically significant differences in the maximum L/N ratio were found

Table 3. The Mean and the Maximum Lesion/Contralateral Normal Brain Tissue Ratio of 68 Patients

	Mean L/N, Median (IQR)	*P* Value	Maximum L/N, Median (IQR)	*P* Value
Age		**0.0321**		**0.0112**
≥65 years	2.82 (2.35–3.15)		4.74 (3.33–5.27)	
<65 years	2.04 (1.60–2.81)		3.13 (2.43–4.57)	
Sex		0.426		0.594
Male	2.37 (1.67–3.00)		3.73 (2.33–4.79)	
Female	2.26 (1.89–3.44)		3.77 (2.66–4.83)	
Contrast enhancement in magnetic resonance imaging		**<0.0001**		**<0.0001**
Yes	2.67 (2.05–3.38)		4.56 (3.25–5.26)	
No	1.60 (0.97–1.94)		2.34 (1.52–2.78)	
World Health Organization grade		**<0.0001**		**<0.0001**
II (n = 28)	1.60 (1.00–2.06)		2.49 (1.59–3.06)	
III (n = 12)	2.26 (1.80–4.20)		3.83 (3.01–5.94)	
IV (n = 28)	3.02 (2.50–3.42)		4.77 (4.35–5.29)	
Histology		**<0.0001**		**<0.0001**
Diffuse astrocytoma (n = 20)	1.20 (0.75–1.86)		2.09 (1.48–2.63)	
IDH mutant (n = 11)	1.08 (0.63–1.65)		2.14 (1.34–2.52)	
IDH wild-type (n = 9)	1.58 (1.02–2.14)		2.04 (1.56–2.83)	
Oligodendroglioma, *IDH* mutant and 1p19q-codeleted (n = 8)	2.03 (1.82–2.35)		3.17 (2.54–3.65)	
Anaplastic astrocytoma (n = 11)	2.06 (1.67–3.32)		3.67 (2.99–5.18)	
IDH mutant (n = 2)	2.85 (2.27–3.44)		4.74 (3.91–5.57)	
IDH wild-type (n = 9)	2.06 (1.81–2.61)		3.67 (2.97–4.58)	
Anaplastic oligodendroglioma, *IDH* mutant and 1p19q-codeleted (n = 1)	6.05 (NA)		8.86 (NA)	
Glioblastoma (n = 28)	3.02 (2.50–3.42)		4.77 (4.35–5.29)	
IDH mutant (n = 3)	2.61 (2.25–2.82)		3.18 (3.08–3.87)	
IDH wild-type (n = 25)	3.02 (2.50–3.46)		4.84 (4.46–5.30)	
IDH status		**0.00162**		**0.00332**
Mutant (n = 26)	1.75 (1.31–2.23)		2.56 (2.2–3.75)	
Wild-type (n = 42)	2.61 (2.05–3.19)		4.51 (2.99–5.12)	
TERT promoter status		**0.0147**		0.0554
Mutant (n = 28)	2.64 (2.04–3.09)		4.11 (3.05–4.85)	
Wild-type (n = 40)	1.92 (1.35–2.81)		3.07 (2.18–4.62)	
IDH/TERT promoter status		**0.00223**		**0.0110**
Group A: *IDH* mutant/*TERT* mutant (n = 9)	2.08 (1.89–2.67)		3.27 (2.55–3.79)	
Group B: *IDH* mutant/*TERT* wild-type (n = 17)	1.68 (0.96–1.80)		2.49 (1.60–3.08)	
Group C: *IDH* wild-type/*TERT* wild-type (n = 23)	2.49 (1.80–3.21)		4.38 (2.73–5.20)	
Group D: *IDH* wild-type/*TERT* mutant (n = 19)	2.92 (2.26–3.14)		4.56 (3.55–4.90)	

P values in bold font are statistically significant.
L/N, lesion/contralateral normal brain tissue; IQR, interquartile range; *IDH*, isocitrate dehydrogenase; *TERT*, telomerase reverse transcriptase.

between groups B and C ($P = 0.0295$), and B and D ($P = 0.00162$) (Figure 3B).

The mean L/N ratio of *IDH* mutant gliomas ($n = 26$) was significantly lower than that of *IDH* wild-type gliomas ($n = 46$) (median, 1.75, IQR, 1.31–2.23 vs. median, 2.61, IQR, 2.05–3.19; $P = 0.00162$) (Figure 4A). The maximum L/N ratio of *IDH* mutant gliomas was also significantly lower than that of *IDH* wild-type gliomas (median, 2.56, IQR, 2.20–3.75 vs. median, 4.51, IQR, 2.99–5.12; $P = 0.00332$) (Figure 4B). The area under the ROC curves of the mean and the maximum L/N ratio were 0.725 and 0.711, respectively (Figure 4C and D). A mean L/N ratio of 2.05 provided the best sensitivity and specificity for distinguishing between *IDH* mutant and *IDH* wild-type gliomas (69.2% and 76.2%, respectively) (Figure 4C). A maximum L/N ratio of 3.92 provided the best sensitivity and specificity for distinguishing between *IDH* mutant and *IDH* wild-type gliomas (76.9% and 64.3%, respectively) (Figure 4D).

The mean L/N ratio of *TERT* promoter mutant gliomas ($n = 28$) was significantly higher than that of *TERT* promoter wild-type gliomas ($n = 40$) (median, 2.64, IQR, 2.04–3.09 vs. median, 1.92, IQR, 1.35–2.81; $P = 0.0147$) (Figure 5A). However, there was no significant difference between patients with *TERT* promoter wild-type and patients with *TERT* promoter mutant in terms of maximum L/N ratio (median, 4.11, IQR, 3.05–4.85 vs. median, 3.07, IQR, 2.18–4.62; $P = 0.0554$) (Figure 5B). The area under the ROC curve was 0.674 for the mean L/N ratio. A mean L/N ratio of 1.88 provided the best sensitivity and specificity for distinguishing between *TERT* promoter wild-type and *TERT* promoter mutant gliomas (50.0% and 89.3%, respectively) (Figure 5C).

Multiple Regression Analysis for Influential Factor on L/N Ratio

Multiple regression analysis showed that pathologic diagnosis was the only influential factor on both mean L/N ratio (P value of F test <0.0001; adjusted $R^2 = 0.435$) and maximum L/N ratio (P value of F test <0.0001; adjusted $R^2 = 0.445$). *IDH* mutation status and contrast enhancement lesion in MRI might influence both mean and maximum L/N ratio, although there were no statistically differences. On the other hand, age, sex, and *TERT* promoter mutation might have little influence on L/N ratio (Table 4).

DISCUSSION

[^{11}C]Methionine PET has been recently recommended for use in the management of glioma.[24] [^{11}C]Methionine accumulates preferentially in tumor but also accumulates in normal brain tissue.[25] Thus, we used the mean and maximum of the L/N ratio, which is the tumor standardized uptake value divided by the mean standardized uptake value of the normal contralateral region of the brain.

Correlation Between L/N Ratio and Pathologic Diagnosis with WHO Grade

In the present study, the L/N ratios of high-grade gliomas were significantly higher than those of low-grade gliomas, which is in agreement with the findings of previously studies.[7,9,11,15,26-29] We also found a positive correlation between the WHO grade and the accumulation of [^{11}C]methionine in PET among astrocytomas, although there was no statistically significant difference between anaplastic astrocytomas and glioblastomas. [^{11}C]Methionine PET

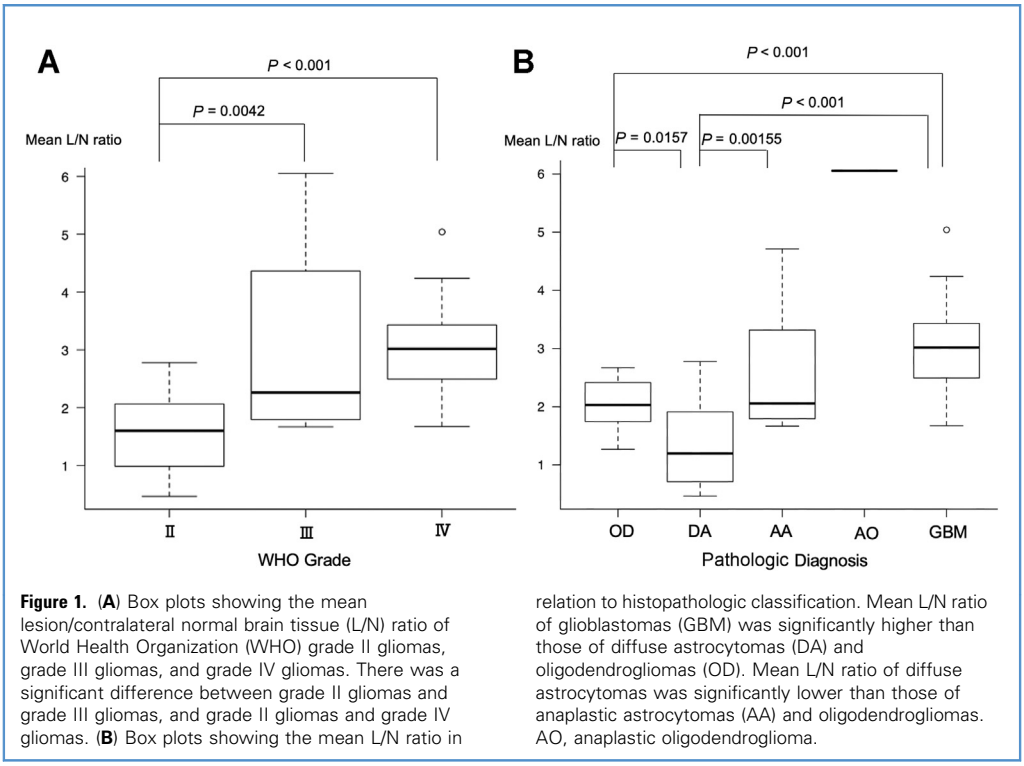

Figure 1. (**A**) Box plots showing the mean lesion/contralateral normal brain tissue (L/N) ratio of World Health Organization (WHO) grade II gliomas, grade III gliomas, and grade IV gliomas. There was a significant difference between grade II gliomas and grade III gliomas, and grade II gliomas and grade IV gliomas. (**B**) Box plots showing the mean L/N ratio in relation to histopathologic classification. Mean L/N ratio of glioblastomas (GBM) was significantly higher than those of diffuse astrocytomas (DA) and oligodendrogliomas (OD). Mean L/N ratio of diffuse astrocytomas was significantly lower than those of anaplastic astrocytomas (AA) and oligodendrogliomas. AO, anaplastic oligodendroglioma.

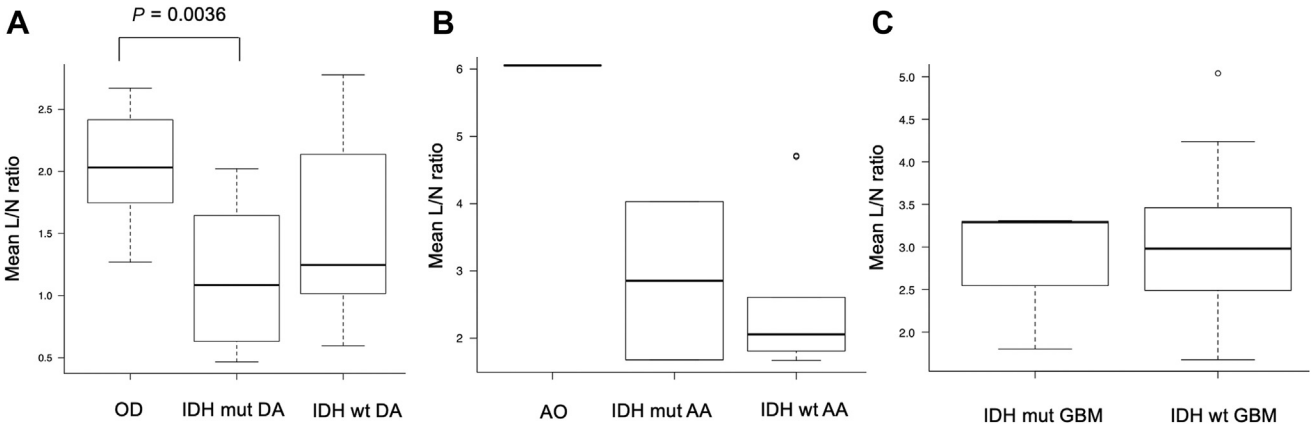

Figure 2. Box plots showing the mean lesion/contralateral normal brain tissue (L/N) ratio in relation to histopathologic and gene status classification according to the revised 2016 World Health Organization classification among grade II (**A**), grade III (**B**), and grade IV (**C**). There was a statistically difference between oligodendrogliomas (OD) and isocitrate dehydrogenase *(IDH)* mutant (mut) diffuse astrocytomas (DA). AA, anaplastic astrocytomas; GBM, glioblastoma; wt, wild-type.

has been widely used to evaluate glioma and several reports have investigated the relationship between the uptake of [^{11}C]methionine using PET and the pathologic diagnosis of glioma based on the morphology. Shinozaki et al.[11] reported that L/N ratio increased significantly as tumor grade advanced in astrocytomas, whereas Hatakeyama et al.[28] found that there were significant differences only between diffuse astrocytomas and anaplastic astrocytomas. Moreover, Kato et al.[30] also found significant differences between glioblastomas and anaplastic astrocytomas/diffuse astrocytomas. However, there is still controversy regarding the accumulation of amino tracer in PET in patients with oligodendroglioma. Shinozaki et al.[11] and Takei et al.[31] reported that among grade II gliomas, the mean L/N ratio in oligodendrogliomas was significantly higher than that in astrocytomas, in agreement with the findings of the present study. Kebir et al.,[32] Kato et al.,[30] and Saito et al.[33] also

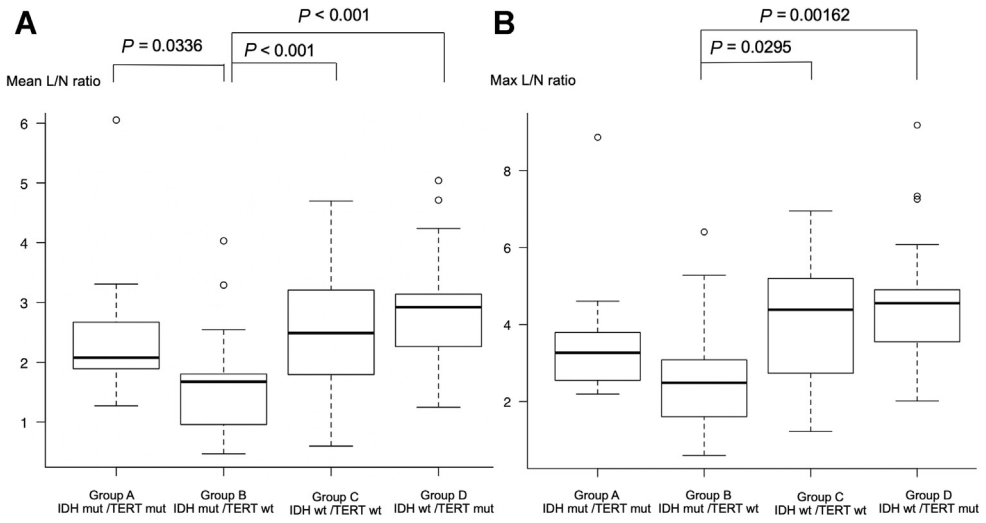

Figure 3. Box plots showing the mean (**A**) and the maximum (**B**) lesion/contralateral normal brain tissue (L/N) ratios in relation to gliomas subgrouping isocitrate dehydrogenase *(IDH)*/telomerase reverse transcriptase (*TERT*) promoter mutation. There were statistically differences in the mean and the maximum L/N ratios between groups B and group C, and groups B and group D. There was a statistically difference in the mean L/N ratio groups A and group B. mut, mutant; wt, wild-type.

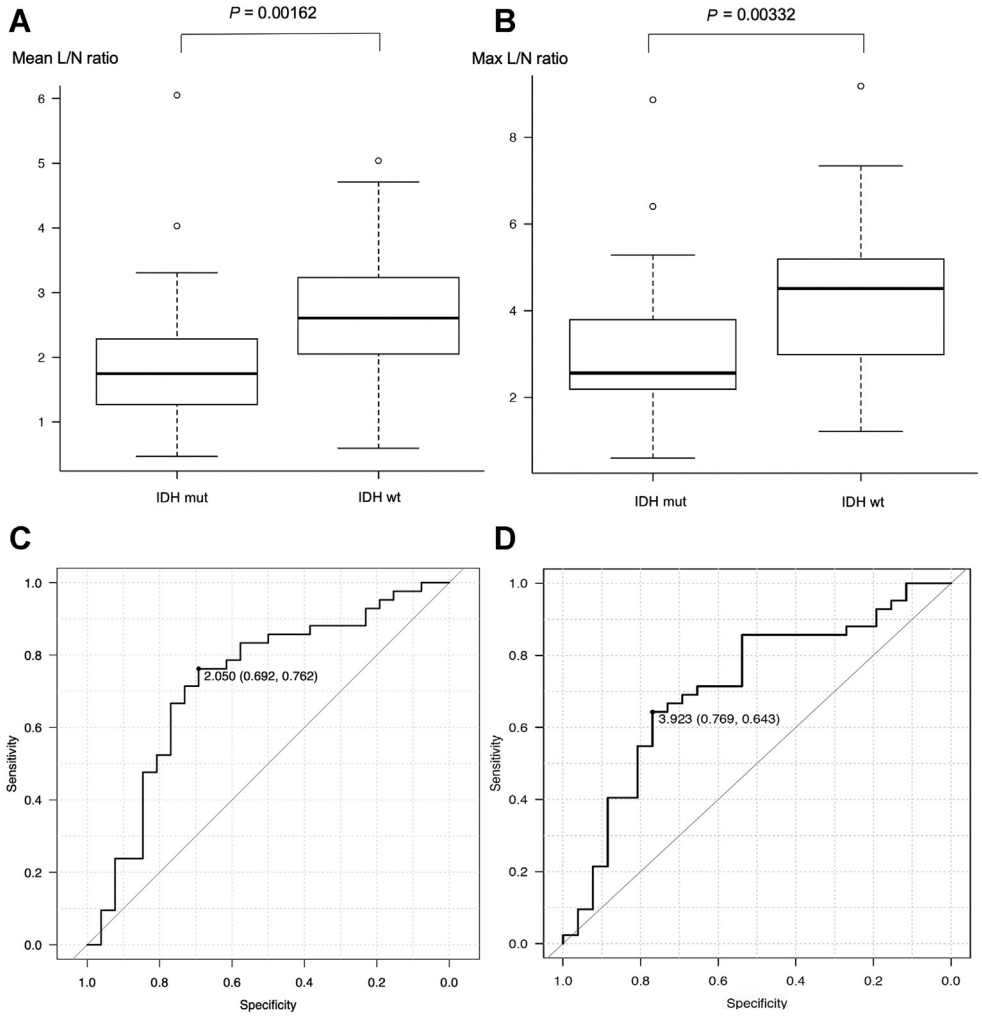

Figure 4. Box plots showing the mean (**A**) and the maximum (**B**) lesion/contralateral normal brain tissue (L/N) ratios in relation to gliomas subgrouping *IDH* mutation. There were statistically differences in the mean and the maximum L/N ratios *IDH* mutant (IDH mut) gliomas and *IDH* wild-type (IDH wt) gliomas. Receiver operating characteristic curve of the mean (**C**) and the maximum (**D**) L/N ratios for differentiating *IDH* mutant gliomas and *IDH* wild-type gliomas. The area under the curve of the mean L/N ratio was larger than that of the maximum L/N ratio with the value of 0. 725 versus 0.711.

reported that among grade II and grade III gliomas, the mean L/N ratio was significantly higher in oligodendrogliomas than in astrocytomas. Okubo et al.[34] reported that the expression of L-type amino acid transporter 1 in the tumor endothelial cells, which is one of the major routes for the transport of [^{11}C] methionine, increased along with glioma grade and was significantly correlated with L/N ratio, probably because of the increased number of microvessels in the tumor. Nojiri et al.[35] reported that an increase in microvessels in oligodendrogliomas correlated with higher [^{11}C]methionine uptake compared with that in astrocytoma. In contrast, Verger et al.[36] concluded that there was no difference in L/N ratios between astrocytomas and oligodendroglioma.

Correlation Between L/N Ratio and IDH Status

Since the revision of the WHO classification of central nervous system tumors in 2016, genetic analysis has become essential for the diagnosis of glioma.[3] In several recent reports regarding the relationship between molecular analysis of gliomas and PET findings,[8,9,21,29,31,32,36-38] all but one[21] concluded *IDH* wild-type gliomas showed a significantly higher accumulation of amino tracer than did *IDH* mutant gliomas.[8,9,29,31,32,36-38] In the present study, the mean and maximum L/N ratios were significantly higher in the *IDH* wild-type gliomas than in the *IDH* mutant gliomas. In addition, a mean L/N ratio of 2.05 provided the best sensitivity and specificity for distinguishing between *IDH* mutant and *IDH* wild-type gliomas (69.2% and 76.2%, respectively; area

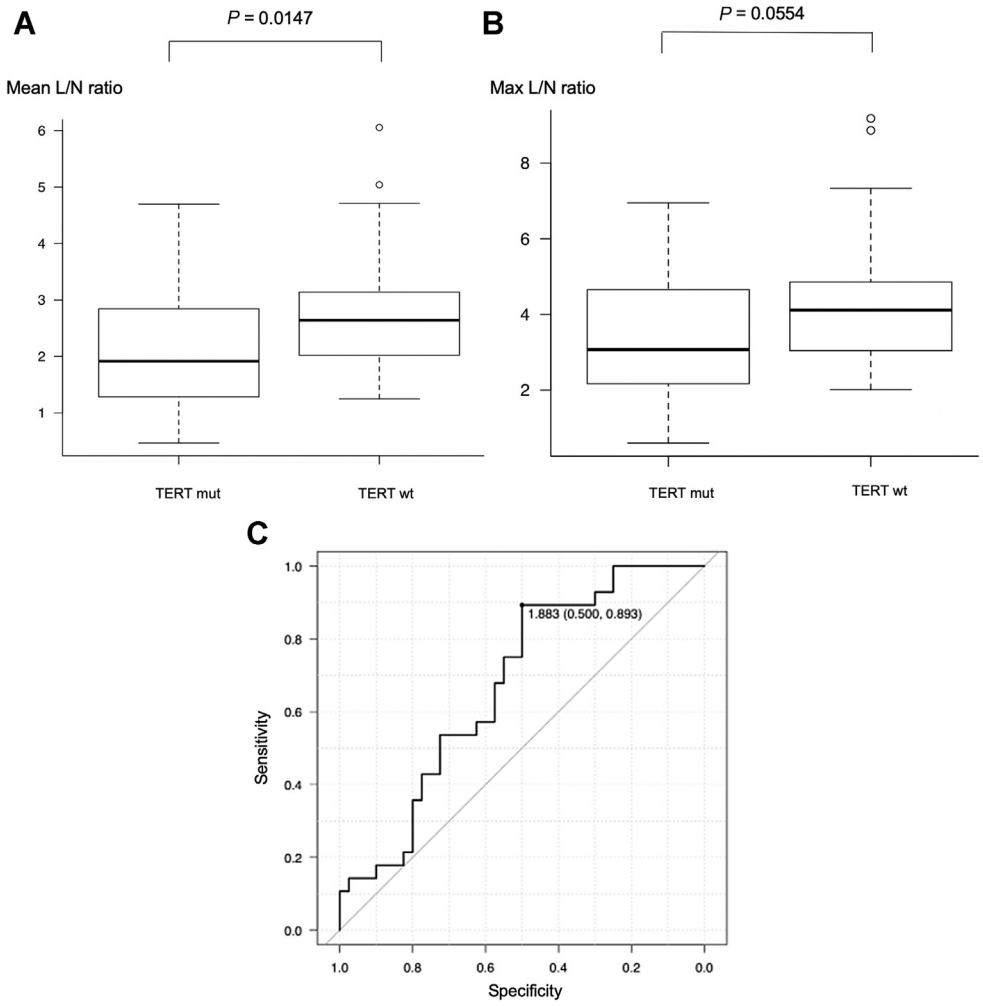

Figure 5. Box plots showing the mean (**A**) and the maximum (**B**) lesion/contralateral normal brain tissue (L/N) ratios in relation to gliomas subgrouping TERT promoter mutation. There were statistically differences in the mean L/N ratio between *TERT* promoter mutant (TERT mut) gliomas and wild-type (TERT wt) gliomas. Receiver operating characteristic curve of the mean L/N ratio for differentiating *TERT* promoter mutant gliomas and *TERT* promoter wild-type gliomas. The area under the curve of the mean L/N ratio was 0.674 (**C**).

under the curve [AUC], 0.725). Takei et al.[31] reported that when the cutoff value of the mean L/N ratio of [^{11}C]methionine was set at 2.69, the sensitivity and specificity were 71.8% and 92.2%, respectively, and the AUC was 0.877. These investigators also reported[31] that [^{11}C]choline PET provided more precise diagnosis and could distinguish between the *IDH* mutant gliomas and the *IDH* wild-type gliomas, with AUC of 0.906. Verger et al. also reported that the usefulness of [^{18}F]fluoroethyl-L-tyrosine PET. These investigators concluded that the combined mean L/N ratio and time from the beginning of the dynamic acquisition up to the maximum uptake of amino tracer in the lesion achieved an accuracy of 73% in predicting *IDH* status.[36] The maximum L/N ratio of [^{11}C]methionine PET has also been considered useful for distinguishing between the *IDH* mutant gliomas and the *IDH* wild-type gliomas. Ogawa et al.[37] reported that a cutoff value of 3.724 provided the best sensitivity and specificity (51.7% and 88.5%, respectively; AUC, 0.727). These investigators also reported that the AUC of the L/N ratio using the [^{18}F]fluoroethyl-L-tyrosine PET was significantly higher than that for [^{11}C]methionine PET.[37] In the present study, a maximum L/N ratio of 3.92 provided the best sensitivity and specificity for distinguishing between *IDH* mutant and *IDH* wild-type gliomas (76.9% and 64.3%, respectively; AUC, 0.711). However, the mean L/N ratio provided a more precise diagnosis than maximum L/N ratio for predicting *IDH* status. Although multiple regression analysis showed that pathologic diagnosis was the only influential

Table 4. Multivariate Regression Analysis of 67 Patients

	Mean L/N Ratio					Maximum L/N Ratio				
	Partial Regression Coefficient	95% CI	VIF	*t* Value	*P* Value	Partial Regression Coefficient	95% CI	VIF	*t* Value	*P* Value
Age: <65 versus ≥65 years	−0.147	−0.663–0.369	1.206	−0.571	0.570	0.020	−0.802 −0.842	1.206	0.049	0.961
Sex: male versus female	0.122	−0.306–0.551	1.059	0.571	0.570	0.089	−0.594 −0.772	1.059	0.261	0.795
Contrast enhancement in MRI: no versus yes	0.442	−0.179–1.063	1.491	1.425	0.160	0.871	−0.119 −1.861	1.491	1.762	0.083
Histology			1.270					1.270		
Diffuse astrocytoma	Reference									
Oligodendroglioma	0.874	0.024–1.723		2.059	**0.044**	1.369	0.015 −2.723		2.023	**0.048**
Anaplastic astrocytoma	0.929	0.225–1.632		2.643	**0.011**	1.318	0.197 −2.440		2.353	**0.022**
Glioblastoma	1.236	0.569–1.904		3.710	**0.0005**	1.944	0.881 −3.008		3.661	**0.0005**
IDH status: mutant versus wild-type	0.448	−0.124–1.020	1.415	1.567	0.123	0.538	−0.374 −1.450	1.415	1.181	0.243
TERT promoter status: mutant versus wild-type	0.0317	−0.471–0.534	1.259	0.126	0.900	0.134	−0.666 −0.935	1.259	0.336	0.738

P values in bold font are statistically significant.
L/N, lesion/contralateral normal brain tissue; CI, confidence interval; VIF, variance inflation factor.

factor on both mean L/N ratio and maximum L/N ratio in the current study, predicting the IDH mutation status in newly diagnosed gliomas noninvasively before tumor resection was meaningful in deciding surgical strategy.

TERT Promoter Mutation and ATRX Alteration in Gliomas

In the revised 2016 WHO classification of central nervous system tumors, *IDH* wild-type gliomas are still fuzzy because they contain diffuse astrocytoma, anaplastic astrocytoma, and glioblastoma. Astrocytomas are defined only by the presence of *IDH* mutation, whereas oligodendrogliomas are defined by the presence of *IDH* mutation and 1p/19q codeletion; thus, further molecular markers are necessary to assess the precise prognosis, particularly in categorizing *IDH* wild-type astrocytomas. Oligodendroglioma is frequently accompanied by *IDH* mutation, *TERT* promoter mutation, and 1p/19q codeletion. ATRX (α thalassemia/mental retardation syndrome X-linked) gene alteration is common in diffuse astrocytoma and secondary glioblastoma, and *TERT* promoter mutation is frequently seen in oligodendroglioma and primary glioblastoma.[39,40] Eckel-Passow et al.[41] categorized gliomas based on 1p/19q codeletion, *IDH* mutation, and *TERT* promoter mutation and Pekmezci et al.[42] categorized gliomas based on 1p/19q codeletion, *IDH* mutation, *ATRX* alteration, and *TERT* promoter mutation. Arita et al.[43] also classified patients with glioma into 4 groups according to the *IDH* and *TERT* promoter status. The common result of these previous studies is that gliomas with *TERT* promoter mutation with *IDH* mutation have a better prognosis, whereas those with *TERT* promoter mutation without *IDH* mutation have a worse prognosis.[42,43] Therefore, *TERT* promoter status may add prognostic value in the management of glioma.

Correlation Between L/N Ratio and TERT Promoter Status

Regarding *TERT* promoter, only 1 study has investigated the relationship between the uptake of the amino tracer and *TERT* promoter mutation.[9] Unterrainer et al.[9] used [^{18}F]GE-180 PET in both newly diagnosed and recurrent gliomas and stated that there was no association between uptake intensity and *TERT* promoter mutation. To the best of our knowledge, the present study is the first report of L/N ratio of [^{11}C]methionine and the status of *TERT* promoter mutation in the newly diagnosed and untreated gliomas. In the present study, the mean L/N ratios of *TERT* promoter wild-type gliomas were significantly lower than those of *TERT* promoter mutant gliomas, but there was no statistically significant difference in terms of maximum L/N ratio. This situation is probably because *TERT* promoter mutation is seen frequently in oligodendroglioma and primary glioblastoma, in which accumulation of amino tracer in the tumor is greater compared with that in lower-grade astrocytomas.[15,28,30,32]

Correlation Between L/N Ratio and IDH/TERT Promoter Status

In the present study, there were statistically significant differences for both the mean and maximum L/N ratios between group B (*IDH* mutant/*TERT* promoter wild-type) and C (*IDH* wild-type/*TERT* promoter wild-type), and also between group B (*IDH* mutant/*TERT* promoter wild-type) and group D (*IDH* wild-type/*TERT* promoter mutant). This situation is probably because group B comprised mostly patients with diffuse astrocytoma, and the proportion of high-grade patients with glioma increased in the order of group B, group C, and group D. There were also statistically significant differences in the mean L/N ratio between group A (*IDH* mutant/*TERT* promoter mutant) and group B (*IDH* mutant/*TERT* promoter wild-type). In group A, about 90% of tumors were oligodendrogliomas, whereas about 70% of tumors in group B were low-grade gliomas (mainly diffuse astrocytomas). As shown in **Figure 2**, the mean L/N ratio was higher in oligodendrogliomas than in diffuse astrocytomas. In the present study, multiple regression analysis showed that *IDH* status had more impact on L/N ratio than did *TERT* promoter status.

Limitations

There are some limitations to this study. First, the relatively small number of patients might influence the analysis. Second, there was inconsistency in the timing between evaluation with [^{11}C] methionine PET and tumor resection, which could possibly have influenced the L/N ratio.

CONCLUSIONS

Distinguishing glioma subtypes based on the revised 2016 WHO classification of the central nervous system tumors on the basis of [^{11}C]methionine PET alone seems to be difficult. Although multiple regression analysis showed that pathologic diagnosis was the only influential factor on L/N ratio, the present finding that L/N ratio of [^{11}C]methionine was significantly higher in *IDH* wild-type gliomas than in *IDH* mutant gliomas indicates that [^{11}C] methionine PET may be a useful and noninvasive technique for predicting *IDH* mutation status in newly diagnosed and untreated gliomas before tumor resection.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Kosuke Nakajo: Conceptualization, Investigation, Writing - original draft. **Takehiro Uda:** Investigation, Supervision. **Toshiyuki Kawashima:** Writing - original draft. **Yuzo Terakawa:** Investigation, Supervision. **Kenichi Ishibashi:** Investigation, Supervision. **Naohiro Tsuyuguchi:** Conceptualization, Investigation, Supervision. **Yuta Tanoue:** Writing - original draft. **Atsufumi Nagahama:** Writing - original draft. **Hiroshi Uda:** Writing - original draft. **Saya Koh:** Writing - original draft. **Tsuyoshi Sasaki:** Writing - original draft. **Kenji Ohata:** Supervision. **Yonehiro Kanemura:** Investigation, Resources, Supervision. **Takeo Goto:** Supervision.

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