

Diagnostic value of PET/CT with ¹¹C-methionine (MET) and ¹⁸F-fluorothymidine (FLT) in newly diagnosed glioma based on the 2016 WHO classification

Tomoya Ogawa

Kagawa University

Nobuyuki Kawai (✉ nobu@kagawa-reha.net)

Kagawa Rehabilitation Hospital <https://orcid.org/0000-0002-3293-6917>

Keisuke Miyake

Kagawa University

Aya Shinomiya

Kagawa University

Yuka Yamamoto

Kagawa University

Yoshihiro Nishiyama

Kagawa University

Takashi Tamiya

Kagawa University

Original research

Keywords: ¹¹C-methionine, ¹⁸F-fluorothymidine, Glioma, IDH1 mutation, PET/CT

Posted Date: February 28th, 2020

DOI: <https://doi.org/10.21203/rs.2.24837/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at EJNMMI Research on May 7th, 2020. See the published version at <https://doi.org/10.1186/s13550-020-00633-1>.

Abstract

Background

The molecular features of *isocitrate dehydrogenase (IDH)* mutation and chromosome 1p and 19q (1p/19q) codeletion status have pivotal role for differentiating gliomas and have been integrated in the World Health Organization (WHO) classification in 2006. Positron emission tomography (PET) with ^{18}F -fluorothymidine (FLT) has been shown to evaluate tumour grade and proliferative activity more accurately than L-methyl- ^{11}C -methionine (MET) in glioma patients. Herein, we compared tracer uptakes of MET-PET/CT and FLT-PET/CT for differentiating glioma according to the 2016 WHO classification especially in relation to *IDH1* mutation status.

Methods

In total, 81 patients with newly diagnosed supratentorial glioma were enrolled in this study. They underwent PET/CT studies with MET and FLT before surgery. The molecular features and histopathological diagnosis based on the 2016 WHO classification were determined using surgical specimens. The ratios of the maximum standardized uptake value (SUV) of the tumours to the mean SUV of the contralateral cortex (T/N ratios) were calculated on MET-PET/CT and FLT-PET/CT images.

Results

The mean T/N ratios of MET-PET/CT and FLT-PET/CT in *IDH1*-wildtype tumours were significantly higher than those in *IDH1*-mutant tumours ($P < 0.001$ and $P < 0.001$, respectively). Receiver operating characteristic analysis for differentiating *IDH1* mutation status showed that the area under the curve of the FLT T/N ratio was significantly larger than that of the MET T/N ratio ($P < 0.01$). The mean T/N ratio of FLT-PET/CT in *IDH1*-wildtype tumours was significantly higher than that in *IDH1*-mutant tumours among grade ≥ 3 and ≥ 4 gliomas ($P = 0.005$), but this was not the case for MET-PET/CT. Both MET-PET/CT and FLT-PET/CT were able to distinguish between grade ≥ 3 and ≥ 4 gliomas in *IDH1*-mutant tumours ($P = 0.002$ and $P < 0.001$, respectively), but only FLT-PET/CT was able to distinguish between grade ≥ 3 and ≥ 4 gliomas in *IDH1*-wildtype tumours ($P = 0.029$).

Conclusion

This study showed that FLT-PET/CT can be used to determine the *IDH1* mutation status and evaluate glioma grade more accurately than MET-PET/CT. The characteristics of FLT-PET/CT can overcome the weakness of MET-PET/CT for differentiating gliomas according to the 2016 WHO classification.

Introduction

The updated 2016 edition of the World Health Organization (WHO) Classification of Tumours of the Central Nervous System (CNS) uses molecular parameters and histology to define the main tumours categories for the first time [1]. This represents a shift from the traditional way of using neuropathological

diagnoses primarily based on microscopic features, to using biologically oriented diagnoses. Among several molecular features, isocitrate dehydrogenase (IDH) mutation and chromosome 1p and 19q (1p/19q) codeletion status are the genetic alterations that have had a significant impact on the new tumour classification [2]. Several studies have shown that IDH1 mutation is a strong prognostic maker, and this may well be the most upstream genetic event in the tumourigenesis and may drive other genetic changes in tumour cells [3, 4].

Positron emission tomography (PET) with L-methyl-¹¹C-methionine (MET) has been widely used as an imaging tool for brain tumour detection, tumour grading, and prediction of prognosis in patients with gliomas [5]. Recent studies have examined MET uptake for differentiating glioma based on the 2016 WHO classification especially in relation to IDH1 mutation and 1p/19q codeletion status. In general, MET uptakes in IDH1-wildtype gliomas were significantly higher compared with those in IDH1-mutant gliomas [6, 7, 8]. However, several amino acid PET studies showed paradoxically higher tracer uptakes in IDH-mutant gliomas, especially with oligodendroglial components, compared with counterpart IDH1-wildtype tumours [8, 9, 10]. These results suggest that amino acid PET studies are not fully consistent or accurate for differentiating glioma at diagnosis based on the 2016 WHO classification.

3'-deoxy-3'-[¹⁸F]fluorothymidine (FLT), a fluorinated thymidine analog, has emerged as a promising PET tracer for evaluating tumour proliferating activity in various malignant tumours. FLT allows the direct measurement of cellular thymidine kinase-1 (TK1), which has been reported to be proportional to the proliferation activity of a tumour [11, 12]. As FLT uptake in normal brain tissue is very low, FLT-PET provides a low-background brain image and thus is considered useful for imaging brain tumours. FLT-PET has been found to be useful for assessing the proliferative activity of gliomas in vivo [13, 14, 15]. Previously, we reported that FLT-PET was superior to MET-PET in non-invasive tumour grading and assessment of proliferative activity in gliomas of different grades based on the 2007 WHO classification [13].

Despite the reported superiority of FLT-PET for accurate diagnosis based on the 2007 WHO classification, no study has evaluated the usefulness of FLT-PET for glioma diagnosis based on the revised 2016 WHO classification. In the present study, we retrospectively analysed MET-PET/CT and FLT-PET/CT in newly diagnosed gliomas for differentiating glioma according to the 2016 WHO classification especially in relation to IDH1 mutation status.

Materials And Methods

Patients

From April 2009 to March 2019, 81 patients with newly diagnosed supratentorial glioma (36 men and 45 women; median age 62 years, range 21-86 years) were retrospectively enrolled in this study. All patients underwent routine magnetic resonance imaging (MRI) examinations including gadolinium (Gd)-enhanced T1-weighted MRI and PET studies with MET and FLT within a short period before surgery. Histopathology

including *immunohistochemistry (IHC)* was performed on tissue specimens obtained by biopsy or resection using a multimodal navigation system (StealthStation, Medtronic-Sofamor Danek) by integrating PET/CT images with anatomical MR images. All gliomas were classified or reclassified using the 2016 WHO classification. The presence of *IDH1 mutation was assessed by IHC to detect IDH1 R132H (codon 132 of the IDH1 gene) protein expression. IDH1 sequencing was performed when the IHC studies were negative. The presence or absence of 1p/19q codeletion was determined by fluorescence in situ hybridization analysis. The 1p/19q codeletion status is another important genetic alteration in the 2016 WHO classification. The presence of both the IDH1 mutation and 1p/19q codeletion characterizes oligodendrogliomas, whereas the presence of the IDH1 mutation without 1p/19q codeletion is indicative of astrocytomas [1].*

The patients' characteristics, including histopathological and genetic diagnoses according to the 2016 WHO classification were summarized in Table 1: grade II astrocytoma (diffuse astrocytoma, DA; n=9), grade II oligodendroglioma (OD; n=3), grade III astrocytoma (anaplastic astrocytoma, AA; n=14), grade III oligodendroglioma (anaplastic oligodendroglioma, AO; n=10), grade IV astrocytoma (glioblastoma, GBM; n=45). IDH1 mutation was found in 29 cases (35.8%: 11 grade II, 16 grade III, and 2 grade IV) and 52 cases were classified as IDH1-wildtype (64.2%: 1 grade II, 8 grade III, and 43 grade IV). All grade II and III oligodendrogliomas had both IDH1 mutation and 1p/19q codeletion.

MET and FLT synthesis and PET acquisition

The use of MET and FLT as a PET/CT tracer was approved by the Kagawa University Faculty of Medicine Human Subjects Ethical Committee, and written informed consent was obtained from all patients before PET/CT examinations.

MET and FLT were produced using an HM-18 cyclotron (Sumitomo Heavy Industries Ltd., Tokyo, Japan). PET examinations were performed using a Biograph mCT64 PET/CT scanner (Siemens/CTI, Knoxville, TN, USA). The imaging systems enabled simultaneous acquisition of 74 transverse per field of view (FOV), with an intersection spacing of 3 mm, for a total axial FOV of 21.6 cm. The in-plane transverse reconstructed resolution was 4.3 mm full-width at half maximum (FWHM) in the brain FOV. No special dietary instructions were given to the patients before the PET/CT examinations. Images were acquired with patients resting in the supine position with their eyes closed. CT data were acquired first (tube rotation time, 0.6 sec per revolution; 120 kV; 192 mAs; reconstructed slice thickness of 3 mm) and used for attenuation correction and anatomical localization of the tumours. For MET-PET/CT, a dose of 182-448 MBq (mean, 316 ± 55.6 MBq) of MET was injected intravenously and regional emission images were obtained for 5 min, beginning 10 min after MET administration. For FLT-PET/CT, a dose of 141-398 MBq (mean, 302 ± 45.2 MBq) of FLT was injected intravenously and regional emission images were obtained for 15 min, beginning 60 min after FLT administration. Image reconstruction was performed using ordered subset expectation maximization (OSEM) with time of flight (TOF) and point spread function (PSF). The reconstruction parameters were 2 iterations and 21 subsets. The FWHM of the Gaussian filter was 3 mm.

Image analysis

MET and FLT uptakes in brain lesions were semiquantitatively assessed by evaluating the standardized uptake value (SUV). A region of interest (ROI) was set manually by a nuclear medicine physician (YY or YN) who was blind to pathological information on the hottest area of each lesion or its centre located by MRI, if increased MET and FLT uptake was absent. The maximum value of SUV (SUVmax) was regarded as the representative value of each tumour. To calculate the tumour-to-normal tissue count density (T/N) ratios, the ROI was set on the normal brain parenchyma (usually contralateral to normal cortex), and the mean value of SUV (SUVmean) was calculated. The T/N ratio was determined by dividing the SUVmax of the tumour by the SUVmean of the normal brain tissue.

Statistical analysis

The Mann-Whitney *U* test was used to compare the T/N ratios of MET and FLT in relation to *IDH1* mutation and 1p/19q codeletion status. For multiple comparison, the Kruskal-Wallis test was used to compare the T/N ratios of MET and FLT in relation to WHO grade and histopathological classification on pathology. The Steel-Dwass method was used in post hoc analysis. Receiver operating characteristic (ROC) analysis was performed to compare the diagnostic usefulness of T/N ratios of MET and FLT in predicting *IDH1* mutation status. We determined the cutoff value at which the sum of sensitivity and specificity (Youden's index) was the highest. All statistical analyses were performed using EZR version 1.40 (Saitama Medical Center, Jichi Medical University, Saitama, Japan). A *P* value less than 0.05 was considered statistically significant.

Results

Tracer uptake in relation to histological grade (Table 2)

The median MET T/N ratios in grade I (n = 12), grade II (n = 24), and grade III (n = 45) gliomas were 2.51 (interquartile range [IQR] 1.82–3.13), 5.03 (3.58–6.39), and 5.99 (4.64–7.39), respectively. There were significant differences in the median MET T/N ratios between grade I and II gliomas (*P* < 0.001) and grade II and III gliomas (*P* < 0.001), but not between grade I and III gliomas. The median FLT T/N ratios in grade I, grade II, and grade III gliomas were 2.24 (IQR 1.69–2.62), 6.25 (4.59–8.69), and 15.24 (11.11–20.49), respectively. There were significant differences in the median FLT T/N ratios between grade I and III gliomas (*P* < 0.001) and grade II and III gliomas (*P* < 0.001).

Tracer uptake in relation to histopathological classification

The median MET T/N ratios in DA (n = 9), OD (n = 3), AA (n = 14), AO (n = 10), and GBM (n = 45) were 2.03 (IQR 1.83–2.97), 2.86 (2.51–3.23), 4.65 (3.25–5.99), 5.07 (4.72–7.21), and 5.99 (4.64–7.39), respectively. OD was omitted from the statistical analysis because of the small number of cases. There were significant differences of the median MET T/N ratios between DA and AA (*P* = 0.016), DA and AO (*P* = 0.009), and DA and GBM (*P* < 0.001; Fig. 1A). The median FLT T/N ratios in DA, OD, AA, AO and GBM were

2.35 (IQR 1.69–2.60), 2.14 (1.91–3.42), 7.16 (4.77–9.41), 5.82 (4.26–6.79), and 15.24 (11.11–20.46), respectively. There were significant differences in the median FLT T/N ratios between DA and AA ($P = 0.002$), DA and AO ($P = 0.005$), DA and GBM ($P < 0.001$), AA and GBM ($P = 0.002$), and AO and GBM ($P < 0.001$; Fig. 1B).

Tracer uptake in relation to IDH1 mutation status

In all gliomas, the median MET T/N ratios in IDH1-mutant and IDH1-wildtype tumours were 3.6 (IQR 2.84–5.59) and 5.91 (4.57–7.35), respectively. The median MET T/N ratio in IDH1-wildtype tumours was significantly higher than that in IDH1-mutant tumours ($P < 0.001$; Fig. 2A). The median FLT T/N ratio in IDH1-mutant and IDH1-wildtype gliomas were 4.18 (IQR 2.28–6.39) and 14.7 (8.98–20.38), respectively. Again, the median FLT T/N ratio in IDH1-wildtype tumours was significantly higher than that in IDH1-mutant tumours ($P < 0.001$; Fig. 2B).

There was a significant overlap of MET T/N ratios between IDH1-mutant and IDH1-wildtype tumours. On the other hand, the overlap of FLT T/N ratios between IDH1-mutant and IDH1-wildtype tumours was small. ROC analysis for differentiating IDH1-mutant tumours from IDH1-wildtype tumours showed that the area under the curve (AUC) of the FLT T/N ratio (AUC 0.911, 95% CI 0.847–0.975; Fig. 3B) was significantly larger than that of the MET T/N ratio (AUC 0.727, 95% CI 0.607–0.847; Fig. 3A) ($P < 0.01$). When the cutoff value of the FLT T/N ratio in the ROC curve was set at 6.74, the sensitivity for the differential diagnosis was 92.3%, and the specificity was 75.9%. On the other hand, the sensitivity for the differential diagnosis was 88.5%, and the specificity was 51.7% when the cutoff value of the MET T/N ratio was set at 3.72.

In the present study, more than 50% of the gliomas were grade \geq GBMs, and almost all GBMs were IDH1-wildtype (43/45 cases). PET tracer uptake in GBMs is usually higher than that in other grades of gliomas, and this may influence the results in relation to IDH1 mutation status. Therefore, we conducted further examination of the uptake values in 36 grade \geq and \leq gliomas. In this population, the median MET T/N ratios in IDH1-mutant and IDH1-wildtype tumours were 3.59 (IQR 2.87–5.28) and 5.14 (3.97–6.06), respectively. There was no significant difference in the median MET T/N ratios between IDH1-mutant and IDH1-wildtype tumours (Fig. 4A). In the same population, the median FLT T/N ratios in IDH1-mutant and IDH1-wildtype tumours were 3.43 (IQR 2.20–5.82) and 7.56 (6.00–12.94), respectively. The median FLT T/N ratio in IDH1-wildtype tumours was significantly higher than that in IDH1-mutant tumours ($P < 0.01$; Fig. 4B).

Tracer uptake in relation to histological grade in IDH1-mutant and IDH1-wildtype tumours

In the present study, there were only 2 IDH1-mutant GBMs among the 29 IDH1-mutant tumours. We therefore excluded the 2 IDH1-mutant GBMs from the analysis. In the remaining 27 IDH1-mutant tumours, the median MET T/N ratios were 2.86 (IQR 1.95–3.28) and 4.83 (3.48–6.39) for the grade \geq ($n = 11$) and \leq ($n = 16$) gliomas, respectively. The median MET T/N ratio in the grade \geq gliomas was significantly higher than that in the grade \leq gliomas ($P = 0.002$; Fig. 5A). In the same population, the median FLT T/N ratios in

the grade II and III gliomas were 2.14 (IQR 1.69–2.64) and 5.36 (3.90–7.09), respectively. Again, the median FLT T/N ratio in the grade II gliomas was significantly higher than that in the grade III gliomas ($P < 0.001$; Fig. 5B).

In the 52 IDH1-wildtype tumours, there was only 1 IDH1-wildtype DA. We therefore excluded the DA from the analysis. In the remaining 51 IDH1-wildtype tumours, the median MET T/N ratios were 5.41 (IQR 4.11–6.20) and 6.19 (4.63–7.41) for the grade II ($n = 8$) and III ($n = 43$) gliomas, respectively. There was no significant difference of the median MET T/N ratios between grade II and III gliomas (Fig. 6A). In the same population, the median FLT T/N ratios in the grade II and III gliomas were 8.10 (IQR 6.58–14.53) and 15.65 (10.35–20.77), respectively. The median FLT T/N ratio in the grade II gliomas was significantly higher than that in the grade III gliomas ($P = 0.029$; Fig. 6B).

Discussion

IDH1 mutation status is the genetic alteration with the most significant impact on the updated 2016 edition of the WHO Classification of Tumours of the CNS [1, 2]. Patients with IDH1-mutant astrocytomas have a better overall prognosis compared with those with IDH1-wildtype astrocytomas, even after controlling for histologic grade [1, 3]. Given their distinct molecular origins, the optimal treatment strategies for IDH1-mutant versus IDH1-wildtype tumors should be different. Recently, several studies have reported that response and benefit to treatment differ depending on the IDH1 mutation status [16, 17, 18]. Based on these studies, preoperative prediction of genotypes, especially the IDH1 mutation status, is essential to planning tailored treatment strategies including surgical resection and postoperative adjuvant therapy. This is especially the case with WHO grade II and III gliomas [17]. On the other hand, the great majority of WHO grade II astrocytomas falls into the IDH1-wildtype category (more than 90% of cases) [1], which corresponds most frequently to the clinically defined primary GBM resulting in poor prognosis even with intensive treatments.

Based on the 2016 WHO classification, MET-PET studies in patients with newly diagnosed gliomas have reported a correlation between MET uptake and IDH1 mutation status, showing that IDH1-wildtype gliomas had significantly higher MET uptake than IDH1-mutant gliomas [6, 7, 8]. In the present study, the MET uptakes in IDH1-wildtype tumours were also significantly higher than that in IDH1-mutant tumours in all gliomas, but this was not the case among grade II and III gliomas. Although the sensitivity for the differential diagnosis was high (88.5%), the specificity was low (51.7%) using MET-PET/CT. Kim et al. reported that among grade II and III gliomas, IDH1-mutant and 1p/19q-codeleted oligodendrogliomas were more likely to exhibit higher MET uptake, even paradoxically high, compared with the counterpart IDH1-wildtype astrocytomas [8]. They cautioned that MET uptake for glioma grading according to the 2016 WHO classification was more consistent and accurate for IDH1-wildtype tumours than for IDH1-mutant tumours. Previous studies have shown that MET uptake in gliomas with an oligodendroglial component is higher than in astrocytomas, even in low-grade gliomas [13, 19, 20]. High MET uptakes in IDH1-mutant oligodendrogliomas may cause low specificity for the differential diagnosis. In fact, grade II AOs (median 5.07, IQR 4.72–7.21) showed increased MET uptake as high as those in grade III GBMs

(median 6.19, IQR 4.63–7.41), in which most of them were IDH1-wild type tumours in the present study. Moreover, several amino acid PET studies showed paradoxically higher tracer uptakes in IDH-mutant gliomas, especially with oligodendroglial components, and the inability to predict the IDH1 mutation status among grade 2 and 3 gliomas [9, 10]. These results suggest that the use of amino acid PET for differentiating glioma at diagnosis may be useful, but is subject to controversy, and the relationship between amino acid PET uptake, especially MET uptake and IDH mutation status remains to be elucidated in further studies.

3'-deoxy-3'-[¹⁸F]fluorothymidine (FLT), a fluorinated thymidine analog, has emerged as a promising PET tracer for evaluating tumour proliferating activity in various brain tumours. FLT is phosphorylated by TK1, a principle enzyme in the salvage pathway of DNA synthesis, and trapped inside cells. Phosphorylated FLT is resistant to degradation and suitable for imaging with PET. The application of FLT phosphorylation as a marker of cell proliferation is based on the assumption that cellular FLT trapping is a representation of thymidine incorporation into DNA [11, 12]. FLT-PET has been found useful for noninvasive grading and assessment of proliferative activity especially in newly diagnosed gliomas [13, 14, 15]. Moreover, FLT-PET can provide valuable information regarding treatment response and patient prognosis and regarding the recognition of tumour recurrence in gliomas [21, 22]. In our previous study, FLT-PET was found to be likely superior to MET-PET in tumour grading and assessment of proliferative activity in newly diagnosed gliomas of different grades [13]. In the present study, MET-PET/CT could only discriminate DA from other histological types of gliomas, i.e. DA and AA, DA and AO, and DA and GBM. On the other hand, FLT-PET/CT can distinguish all different types of gliomas except AA and AO based on the 2016 WHO classification. Moreover, both PET tracers could distinguish grade 2 gliomas from higher-grade gliomas (AA and GBM), but the differentiation between grade 2 and 3 gliomas, two groups with different prognosis and management [23], was able to obtain only with FLT-PET/CT.

This is the first study to demonstrate a significant correlation between FLT uptake values and IDH1 mutation status in newly diagnosed gliomas. FLT uptakes in IDH1-wildtype tumours were significantly higher than those in IDH1-mutant tumours. ROC analysis showed that FLT-PET/CT can distinguish the IDH1 mutation status more accurately than MET-PET/CT. Histologically, the prognostic differences between grade 2 diffuse astrocytoma and grade 3 anaplastic astrocytoma were significant [24]. However, recent studies have shown that the prognostic difference between IDH-mutant grade 2 diffuse astrocytomas and IDH-mutant grade 3 anaplastic astrocytomas are not as marked [25], suggesting that IDH status is more important than tumour grade. It should be noted that FLT-PET/CT was able to distinguish the IDH1-mutant tumours from wildtype tumors not only in all gliomas, but also among grade 2 and 3 gliomas, but this was not the case for MET-PET/CT. Although the sensitivity for differential diagnosis of IDH1 mutation status in the ROC analysis was high (92.3%) with FLT-PET, the specificity was not satisfactory (75.9%). This number means that one in 4 gliomas with low FLT uptake below the cutoff value (T/N ratio of 6.74) was an IDH1-wildtype tumour that could harbour an aggressive nature in the tumour. Our previous study showed that FLT uptake in tumours without an observed contrast enhancement effect on MR images was low even if the tumours were high-grade gliomas [15]. Later

studies, including from our laboratory, have shown that the major portion of FLT uptake is due to increased transport and influx through the disrupted blood-brain barrier (BBB) [26, 27]. Non-enhancing tumours with an intact BBB showed limited transport of FLT, and tumour malignancy and cell proliferation activity cannot be adequately assessed by FLT-PET [28]. This can result in underestimation of the aggressiveness of the lesion. This problem can be clarified by further analysis of FLT uptake according to the enhancement status. However, the number of non-enhancing IDH1-wildtype high-grade gliomas is limited in the present study. Furthermore, the degree of enhancement status is various even in enhanced tumours. The issue regarding the relatively low specificity of FLT-PET/CT for differentiating IDH1 mutation status should be addressed with more cases in the future.

The present study had several limitations. First, there was selection bias with respect to the relatively small number of patients with low-grade glioma. Patients with low tracer uptakes were suspected of having low-grade gliomas and considered less likely candidates for surgery, especially when they had few or no symptoms. Second, patients with IDH1-wildtype DA (n = 1) and IDH1-mutant GBMs (n = 2) were excluded from the statistical analysis because of the small number of cases. We only compared the tracer uptakes in grade 2 and 3 gliomas with IDH1 mutation and grade 2 and 3 gliomas without IDH1 mutation. Incidences of IDH1-mutant GBM (less than 10%) and IDH1-wildtype DA are rare in the general population [1, 29], and there were too few datasets available for a meaningful analysis in the present study. Third, in addition to the retrospective nature of the present study, no follow-up data were evaluated in the present study, and the findings need to be analysed in relation to prognosis with a long follow-up period.

Conclusions

IDH1-wildtype tumours showed significantly higher FLT uptake than IDH1-mutant tumours. The accuracy for differentiating IDH1 mutation status with FLT-PET/CT was higher than with MET-PET/CT. FLT-PET/CT was able to distinguish the IDH1-mutant tumours from wildtype tumours not only in all gliomas but also in grade 2 and 3 gliomas. Moreover, only FLT-PET/CT was able to distinguish both between grade 2 and 3 gliomas in IDH1-wildtype tumours and grade 2 and 3 gliomas in IDH1-mutant tumours. These characteristics of FLT-PET/CT can overcome the weakness of MET-PET/CT for differentiating gliomas based on the 2016 WHO classification and should be considered to enable the effective use of FLT-PET/CT for tumour grading, treatment planning, and prediction of prognosis.

Declarations

Abbreviations

AA: Anaplastic astrocytoma; **AO:** Anaplastic oligodendroglioma; **AUC:** Area under the curve; **CT:** Computed tomography; **DA:** Diffuse astrocytoma; **DNA:** Deoxyribonucleic acid; **EZR:** Easy R; **FLT:** ¹⁸F-fluorothymidine; **FOV:** Field of view; **FWHM:** Full-width at half maximum; **GBM:** Glioblastoma multiforme; **Gd:** Gadolinium; **IDH1:** *Isocitrate dehydrogenase 1*; **IHC:** *Immunohistochemistry*; **IQR:** *Interquartile range*; **MBq:**

Megabecquerel; **MET**: ^{11}C -methionine; **MRI**: Magnetic resonance imaging; **OD**: Oligodendroglioma; **OSEM**: Ordered subset expectation maximization; **PET**: Positron emission tomography; **PSF**: Point spread function; **ROC**: Receiver operating characteristic; **ROI**: Region of interest; **SUV**: Standardized uptake value; **SUVmax**: Maximum value of SUV; **SUVmean**: Mean value of SUV; **T/N**: Tumour-to-normal; **TK1**: Thymidine kinase-1; **TOF**: Time of flight; **WHO**: World Health Organization

Ethics approval and consent to participate

All procedures involving human participants were performed in accordance with the ethical standards of the institutional research committee and in accordance with the principles of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The use of MET and FLT as a PET tracer was approved by the Kagawa University Faculty of Medicine Human Subjects Ethical Committee, and written informed consent was obtained from all patients before PET examinations.

Consent for publication

Not applicable.

Availability of data and materials

All datasets used during the current study are available from the first authors or corresponding authors on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by a Grant-in-Aid for Scientific Research (B) (18H02913) from Japan Society for the Promotion of Science.

Author's contribution

Conception and design of the study: TO, NK, KM, TT. Immunohistochemistry: AS. PET/CT examination and ROI setting: YY, YN. Data analysis and interpretation: TO, YY, YN. Manuscript preparation: TO, NK. All authors contributed to discussion of results and have read and approved the final manuscript.

Acknowledgement

We gratefully acknowledge the excellent technical supports of PET radiological technologist at our institution.

References

1. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol.* 2016;131(6):803-20.
2. Weller M, Pfister SM, Wick W, Hegi ME, Reifenberger G, Stupp R. Molecular neuro-oncology in clinical practice: a new horizon. *Lancet Oncol.* 2013;14(9):e370-9.
3. Weller M, Weber RG, Willscher E, Riehm V, Hentschel B, Kreuz M, et al. Molecular classification of diffuse cerebral WHO grade II/III gliomas using genome- and transcriptome-wide profiling improves stratification of prognostically distinct patient groups. *Acta Neuropathol.* 2015;129(5):679-93.
4. Suzuki H, Aoki K, Chiba K, Sato Y, Shiozawa Y, Shiraishi Y, et al. Mutational landscape and clonal architecture in grade II and III gliomas. *Nat Genet.* 2015;47(5):458-68.
5. Glaudemans AW, Enting RH, Heesters MA, Dierckx RA, van Rheenen RW, Walenkamp AM, et al. Value of ¹¹C-methionine PET in imaging brain tumours and metastases. *Eur J Nucl Med Mol Imaging.* 2013;40(4):615-35.
6. Lopci E, Riva M, Olivari L, Raneri F, Soffietti R, Piccardo A, et al. Prognostic value of molecular and imaging biomarkers in patients with supratentorial glioma. *Eur J Nucl Med Mol Imaging.* 2017;44(7):1155-1164.
7. Takei H, Shinoda J, Ikuta S, Maruyama T, Muragaki Y, Kawasaki T, et al. Usefulness of positron emission tomography for differentiating gliomas according to the 2016 World Health Organization classification of tumors of the central nervous system. *J Neurosurg.* 2019;16:1-10.
8. Kim D, Chun JH, Kim SH, Moon JH, Kang SG, Chang JH, et al. Re-evaluation of the diagnostic performance of ¹¹C-methionine PET/CT according to the 2016 WHO classification of cerebral gliomas. *Eur J Nucl Med Mol Imaging.* 2019;46(8):1678-1684.
9. Verger A, Metellus P, Sala Q, Colin C, Bialecki E, Taieb D, et al. IDH mutation is paradoxically associated with higher ¹⁸F-FDOPA PET uptake in diffuse grade II and grade III gliomas. *Eur J Nucl Med Mol Imaging.* 2017;44(8):1306-1311.
10. Verger A, Stoffels G, Bauer EK, Lohmann P, Blau T, Fink GR, et al. Static and dynamic ¹⁸F-FET PET for the characterization of gliomas defined by IDH and 1p/19q status. *Eur J Nucl Med Mol Imaging.* 2018;45(3):443-451.
11. Shields AF, Grierson JR, Dohmen BM, Machulla HJ, Stayanoff JC, Lawhorn-Crews JM, et al. Imaging proliferation in vivo with [¹⁸F]FLT and positron emission tomography. *Nat Med.* 1998;4(11):1334-6.
12. Salskov A, Tammisetti VS, Grierson J, Vesselle H. FLT: measuring tumor cell proliferation in vivo with positron emission tomography and 3'-deoxy-3'-[¹⁸F]fluorothymidine. *Semin Nucl Med.* 2007;37(6):429-39.
13. Hatakeyama T, Kawai N, Nishiyama Y, Yamamoto Y, Sasakawa Y, Ichikawa T, et al. ¹¹C-methionine (MET) and ¹⁸F-fluorothymidine (FLT) PET in patients with newly diagnosed glioma. *Eur J Nucl Med Mol Imaging.* 2008;35(11):2009-17.

14. Yamamoto Y, Ono Y, Aga F, Kawai N, Kudomi N, Nishiyama Y. Correlation of 18F-FLT uptake with tumor grade and Ki-67 immunohistochemistry in patients with newly diagnosed and recurrent gliomas. *J Nucl Med*. 2012;53(12):1911-5.
15. Collet S, Valable S, Constans JM, Lechapt-Zalcman E, Roussel S, Delcroix N, et al. [(18)F]-fluoro-L-thymidine PET and advanced MRI for preoperative grading of gliomas. *Neuroimage Clin*. 2015;8:448-54.
16. Beiko J, Suki D, Hess KR, Fox BD, Cheung V, Cabral M, et al. IDH1 mutant malignant astrocytomas are more amenable to surgical resection and have a survival benefit associated with maximal surgical resection. *Neuro Oncol*. 2014;16(1):81-91.
17. Delev D, Heiland DH, Franco P, Reinacher P, Mader I, Staszewski O, et al. Surgical management of lower-grade glioma in the spotlight of the 2016 WHO classification system. *J Neurooncol*. 2019;141(1):223-233.
18. Kawaguchi T, Sonoda Y, Shibahara I, Saito R, Kanamori M, Kumabe T, et al. Impact of gross total resection in patients with WHO grade III glioma harboring the IDH 1/2 mutation without the 1p/19q co-deletion. *J Neurooncol*. 2016;129(3):505-514.
19. Kato T, Shinoda J, Nakayama N, Miwa K, Okumura A, Yano H, et al. Metabolic assessment of gliomas using 11C-methionine, [18F] fluorodeoxyglucose, and 11C-choline positron-emission tomography. *AJNR Am J Neuroradiol*. 2008 Jun;29(6):1176-82.
20. Manabe O, Hattori N, Yamaguchi S, Hirata K, Kobayashi K, Terasaka S, et al. Oligodendroglial component complicates the prediction of tumour grading with metabolic imaging. *Eur J Nucl Med Mol Imaging*. 2015 May;42(6):896-904.
21. Idema AJ, Hoffmann AL, Boogaarts HD, Troost EG, Wesseling P, Heerschap A, et al. 3'-Deoxy-3'-18F-fluorothymidine PET-derived proliferative volume predicts overall survival in high-grade glioma *J Nucl Med*. 2012;53(12):1904-10.
22. Corroyer-Dulmont A, Pérès EA, Géralt AN, Savina A, Bouquet F, Divoux D, et al. Multimodal imaging based on MRI and PET reveals [(18)F]FLT PET as a specific and early indicator of treatment efficacy in a preclinical model of recurrent glioblastoma. *Eur J Nucl Med Mol Imaging*. 2016;43(4):682-94.
23. Weller M, van den Bent M, Hopkins K, Tonn JC, Stupp R, Falini A, et al. EANO guideline for the diagnosis and treatment of anaplastic gliomas and glioblastoma. *Lancet Oncol*. 2014;15(9):e395-403.
24. Ohgaki H and Kleihues P. Population-based Studies on Incidence, Survival Rates, and Genetic Alterations in Astrocytic and Oligodendroglial Gliomas. *J Neuropathol Exp Neurol*. 2005;64:479-489.
25. Olar A, Wani KM, Alfaro-Munoz KD, Heathcock LE, van Thuijl HF, Gilbert MR, et al. IDH Mutation Status and Role of WHO Grade and Mitotic Index in Overall Survival in Grade II-III Diffuse Gliomas. *Acta Neuropathol*. 2015;129:585-596.
26. Jacobs AH, Thomas A, Kracht LW, Li H, Dittmar C, Garlip G, et al. 18F-fluoro-L-thymidine and 11C-methylmethionine as markers of increased transport and proliferation in brain tumors. *J Nucl Med*. 2005;46(12):1948-58.

27. Shinomiya A, Kawai N, Okada M, Miyake K, Nakamura T, Kushida Y, et al. Evaluation of 3'-deoxy-3'-[18F]-fluorothymidine (18F-FLT) kinetics correlated with thymidine kinase-1 expression and cell proliferation in newly diagnosed gliomas. *Eur J Nucl Med Mol Imaging*. 2013;40(2):175-85.
28. Muzi M, Spence AM, O'Sullivan F, Mankoff DA, Wells JM, Grierson JR, et al. Kinetic analysis of 3'-deoxy-3'-¹⁸F-fluorothymidine in patients with gliomas. *J Nucl Med* 2006;47:1612-21.
29. Hasselblatt M, Jaber M, Reuss D, Grauer O, Bibo A, Terwey S. et al. Diffuse astrocytoma, IDH-wildtype: A dissolving diagnosis. *J Neuropathol Exp Neurol*. 2018;77(6):422–425.

Tables

Due to technical limitations, all table files are only available for download from the Supplementary Files section.

Figures

Fig. 1

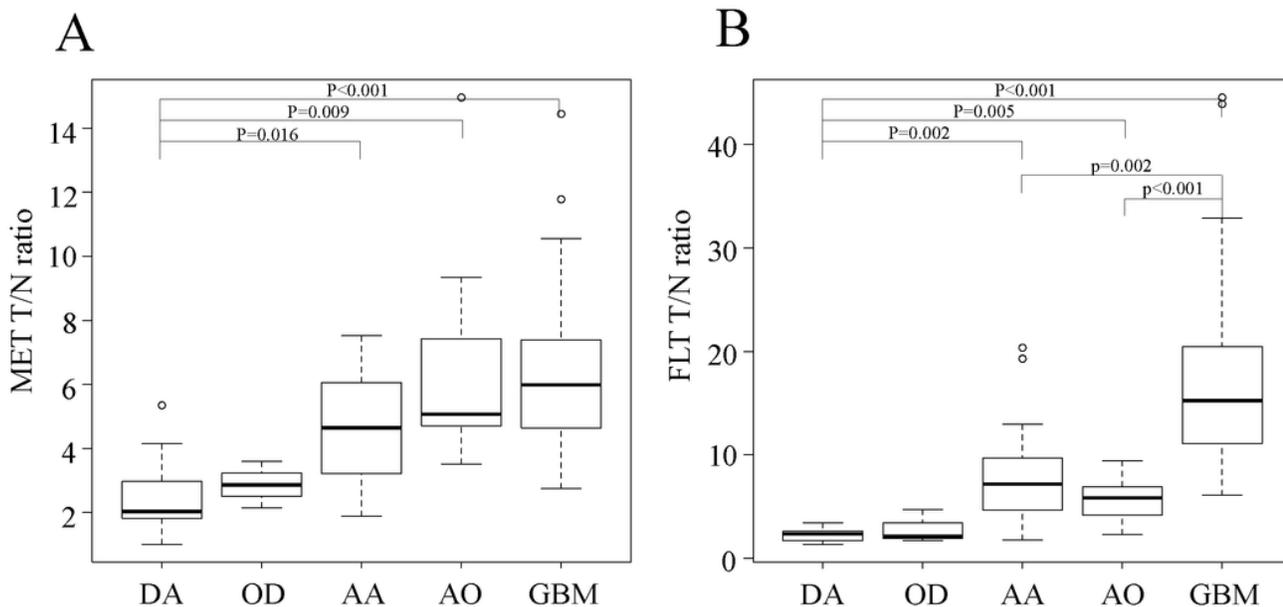


Figure 1

Box plots showing the T/N ratios of MET (A) and FLT (B) in relation to histopathological classification. OD was omitted from the statistical analysis because of small number of cases (n=3). There were significant differences in the median MET T/N ratios between DA and AA (P=0.016), DA and AO (P=0.009), and DA and GBM (P<0.001) (A). There were significant differences in the median FLT T/N

ratios between DA and AA ($P=0.002$), DA and AO ($P=0.005$), DA and GBM ($P<0.001$), AA and GBM, ($P=0.002$) and AO and GBM ($P<0.001$) (B).

Fig. 2

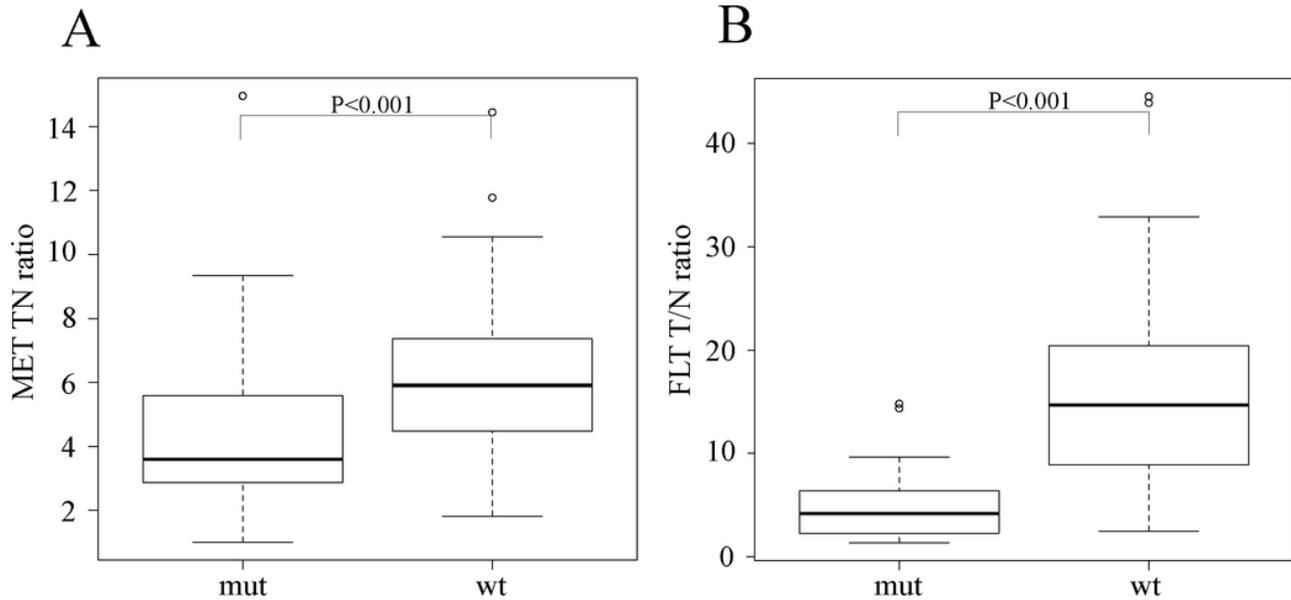


Figure 2

Box plots showing the T/N ratios of MET (A) and FLT (B) in relation to IDH1 mutation status in all gliomas ($n=81$). The tracer uptake in IDH1-wildtype (wt) tumours was significantly higher than that in IDH1-mutant (mut) tumours both in MET-PET ($P<0.001$) and FLT-PET ($P<0.001$).

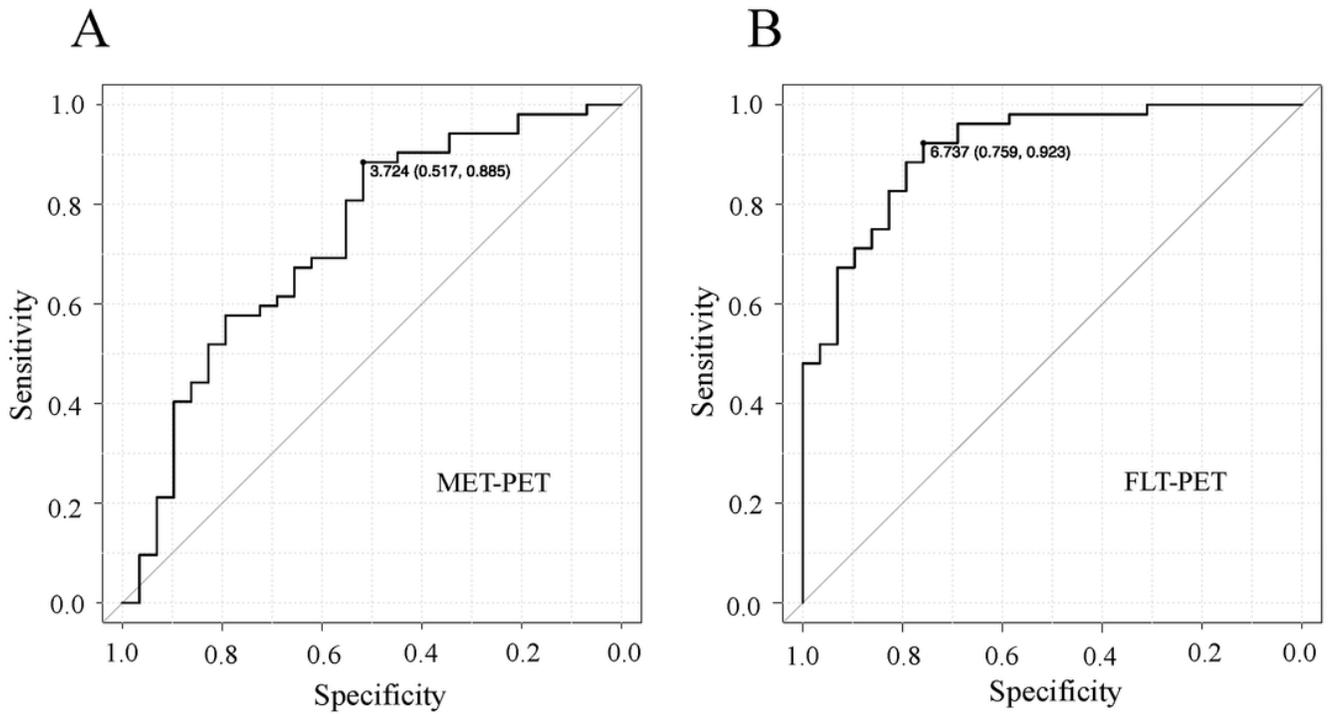


Figure 3

ROC curve of MET (A) and FLT (B) for differentiating IDH1-wildtype from IDH1-mutant tumours in all gliomas (n=81). The area under the curve (AUC) of the FLT T/N ratio (AUC: 0.911, 95% CI 0.847-0.975) was significantly larger than that of the MET T/N ratio (AUC: 0.727, 95% CI 0.607-0.847).

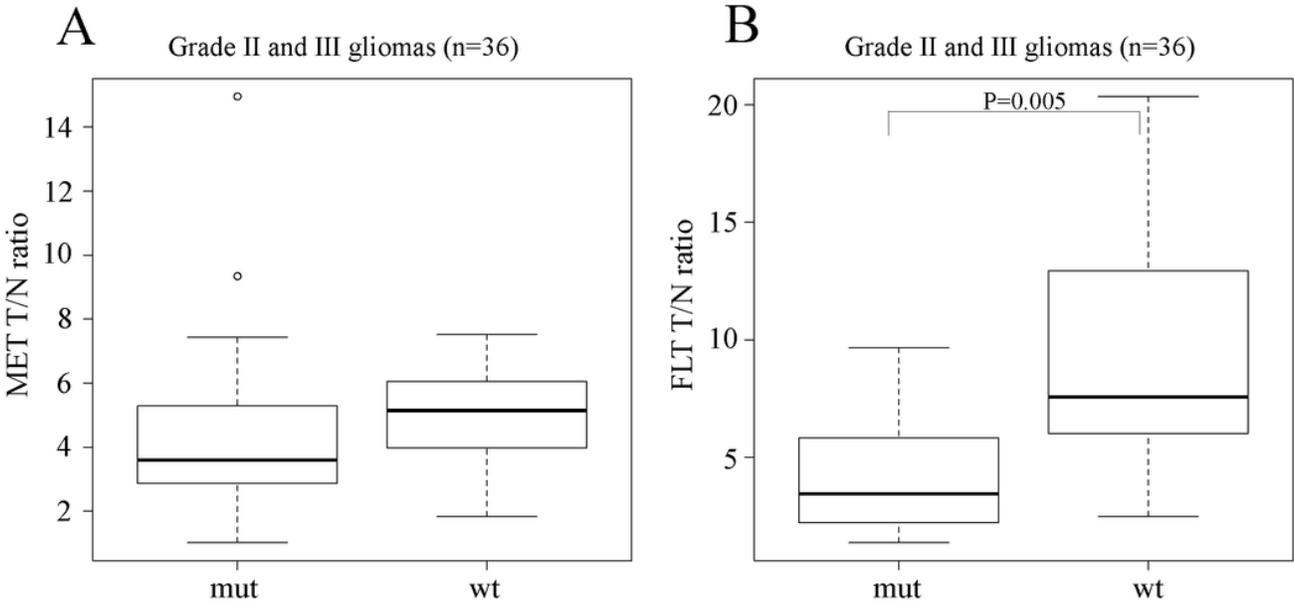


Figure 4

Box plots showing the T/N ratios of MET (A) and FLT (B) in relation to IDH1 mutation status in grade II and III gliomas (n=36). There was no significant difference in the median MET T/N ratios between IDH1-mutant (mut) and IDH1-wildtype (wt) tumours (A). The median FLT T/N ratio in IDH1-wildtype tumours was significantly higher than that in IDH1-mutant tumours (P=0.005) (B).

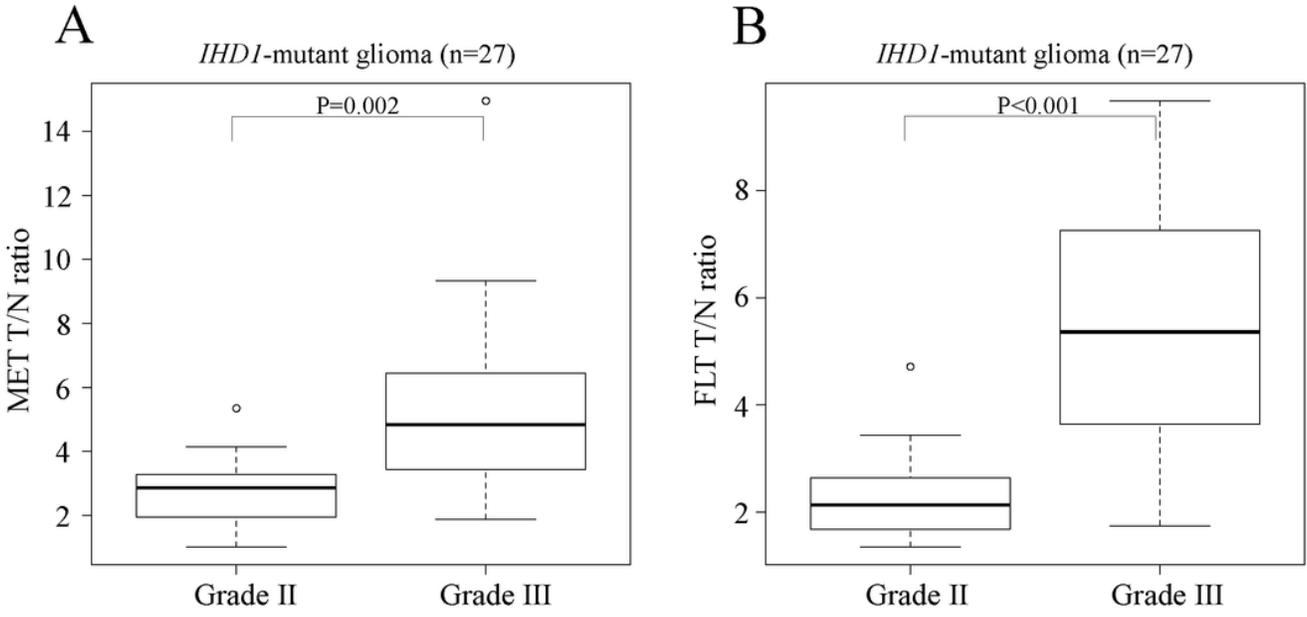


Figure 5

Box plots showing the T/N ratios of MET (A) and FLT (B) in relation to glioma grading in IDH1-mutant tumours (n=27). The tracer uptake in grade III gliomas was significantly higher than that in grade II gliomas both in MET-PET (P=0.002) and FLT-PET (P<0.001).

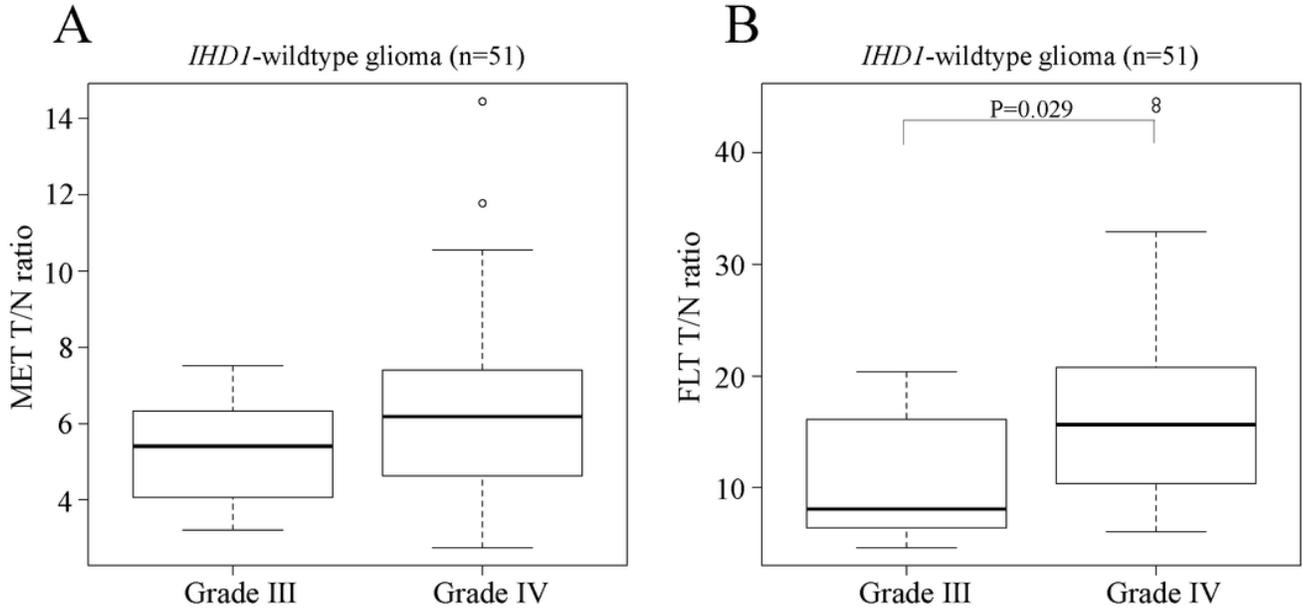


Figure 6

Box plots showing the T/N ratios of MET (A) and FLT (B) in relation to glioma grading in IDH1-wildtype tumours (n=51). There was no significant difference in the median MET T/N ratios between grade III and grade IV gliomas (A). The median FLT T/N ratio in grade IV gliomas was significantly higher than that in grade III gliomas (P=0.029) (B).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table.docx](#)