



Correlation of hypoxia as measured by fluorine-18 fluoroerythronitroimidazole (^{18}F -FETNIM) PET/CT and overall survival in glioma patients

Man Hu^{1,2} · Yufang Zhu³ · Dianbin Mu⁴ · Bingjie Fan² · Shuqiang Zhao⁵ · Guoren Yang⁵ · Li Ma⁵ · Jinsong Zheng⁵ · Jinming Yu^{1,2,6}

Received: 11 June 2019 / Accepted: 14 November 2019 / Published online: 28 November 2019
© Springer-Verlag GmbH Germany, part of Springer Nature 2019

Abstract

Purpose Hypoxia is important in the biology of glioma in humans. Positron emission tomography/computed tomography (PET/CT) with a hypoxia tracer offers a noninvasive method to differentiate individual tumor biology and potentially modify treatment for patients with malignancies. The purpose of this study was to determine whether hypoxia, as measured by fluorine-18 fluoroerythronitroimidazole (^{18}F -FETNIM) PET/CT, was associated with tumor grade, overall survival (OS), and immunohistochemical features related to hypoxia, proliferation, angiogenesis, and the invasion of gliomas.

Procedures Twenty-five patients with gliomas in whom gross maximal resection could be safely attempted were analyzed. All patients underwent ^{18}F -FETNIM PET/CT studies before surgery. The maximum standardized uptake value (SUVmax) was obtained from the PET images of tumor tissues. Tumor specimens were stereotactically obtained for the immunohistochemical staining of hypoxia-inducible factor-1 alpha (HIF-1 α), Ki-67, vascular endothelial growth factor (VEGF), and matrix metalloproteinase 9 (MMP-9).

Results A correlation between the SUVmax and glioma grade was found ($r = 0.881$, $P < 0.001$). The SUVmax was significantly correlated with the expression of HIF-1 α , Ki-67, VEGF, and MMP-9 ($r = 0.820$, 0.747 , 0.606 , and 0.727 ; all $P < 0.001$). Patients with a high SUVmax had significantly worse 3-year OS than those with a low SUVmax (24.4% vs. 82.1%, $P = 0.003$).

Conclusions ^{18}F -FETNIM PET/CT provides an excellent noninvasive assessment of hypoxia in glioma. It can be used to understand the mechanisms by which hypoxia affects the OS of glioma patients.

Keywords Hypoxia imaging · Fluoroerythronitroimidazole · Glioma

The current study received the Merit Award at the 2014 American Society of Clinical Oncology (ASCO) Annual Meeting in an Oral Scientific Session on June 2, 2014, Chicago, Illinois, USA.

This article is part of the Topical Collection on Oncology – Brain

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00259-019-04621-z>) contains supplementary material, which is available to authorized users.

✉ Jinming Yu
sdyujinming@163.com

¹ Shandong University, Jinan, China

² Departments of Radiation Oncology and Shandong Province Key Laboratory of Radiation Oncology, Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Sciences, Jinan, China

³ Departments of Neurosurgery, Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Sciences, Jinan, China

⁴ Department of Pathology, Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Sciences, Jinan, China

⁵ Department of Nuclear Medicine, Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Sciences, Jinan, China

⁶ Jinan, People's Republic of China

Introduction

Glioma is the most common primary brain tumor in adults. The 5-year overall survival (OS) rate is approximately 85% for patients with low grade glioma (LGG), whereas it is less than 5% for those with glioblastoma (GBM) [1]. Hypoxia is a frequent phenomenon and one of the most important biological characteristics in glioma due to inadequate tumor vascularization, the rapid depletion of nutrients, and aberrant growth. Tumor hypoxia in glioma is usually measured using tissue samples obtained by a biopsy or open resection, which is not performed repeatedly because of its invasive nature. Therefore, it is important to establish a noninvasive method to accurately identify tumor hypoxia and its relationship with malignant progression in patients with glioma.

Recently, positron emission tomography/computed tomography (PET/CT) integrated with a hypoxia tracer can serve as a noninvasive technique for detecting tumor hypoxia with individualization and quantification. Among all hypoxia tracers [2–7], limited PET/CT agents have been reported for glioma patients. The most common is fluoromisonidazole (FMISO) [8–12]. The drawbacks of ^{18}F -FMISO, including its lipophilic characteristics and a low target-to-background ratio, have limited its clinical application [13]. PET imaging with the agent 1-(2-hydroxy-1-[hydroxymethyl] ethoxy) methyl-2-nitroimidazole (RP170) was developed to detect the presence of hypoxia in 8 patients with glioma [7]. Fluoroazomycin arabinoside (FAZA) was used to detect hypoxia in high grade glioma (HGG) only in a case report [4], and the potential use of RP170 and FAZA for the clinical detection of hypoxia in brain tumors needs to be further confirmed. Fluoroerythronitroimidazole (FETNIM) is a derivative of nitroimidazole and is more hydrophilic, resulting in lower and more favorable background signals than FMISO [13, 14]. Our previously published data indicated that ^{18}F -labeled FETNIM (^{18}F -FETNIM) PET/CT is a highly promising approach for detecting tumor hypoxia in clinical settings [2, 15, 16]. More importantly, a preclinical biodistribution study showed that ^{18}F -FETNIM levels in the cerebellum were much lower than those in other organs [17]. Based on these findings, we hypothesized that ^{18}F -FETNIM PET/CT could be used to assess hypoxia in glioma patients and provide more information on biological tumor characteristics.

The purpose of this study was to determine whether ^{18}F -FETNIM uptake, as analyzed by PET/CT, could detect tumor hypoxia and the association of ^{18}F -FETNIM uptake with malignant progression, including tumor grade, OS, and immunohistochemical features related to hypoxia, proliferation, angiogenesis, and invasion for patients with gliomas.

Patients and methods

Patients

Between August 2011 and October 2015, glioma patients in whom gross maximal resection could be safely attempted were enrolled. Before any treatment, all patients received standard presurgery evaluations, including contrast-enhanced magnetic resonance imaging (MRI) of the brain. MRI scanning was performed with a 3.0T MR scanner (Philips Achieva 3.0T). The scanning protocol parameters were as follows: axial T1-weighted imaging (repetition time (TR) = 495 ms, echo time (TE) = 10 ms, slice thickness/gap = 3 mm/0 mm, matrix = 512, number of signals averaged (NSA) = 1, field of view (FOV) = 340 mm × 340 mm), and axial T2-weighted imaging (TR = 4213 ms, TE = 120 ms, slice thickness/gap = 3 mm/0 mm, matrix = 512, NSA = 1, FOV = 340 mm × 340 mm). Patients with a previous history of malignant disease (excluding glioma), contraindication of surgical operations, and pregnancy were excluded. The diagnoses were confirmed by the final histological features after surgery. All tumors were graded according to the 2007 World Health Organization (WHO) classification of tumors of the central nervous system.

This study was approved by the Institutional Review Board and the Administration of Radioactive Substances Advisory Committee of Shandong Cancer Hospital and Institute. Written informed consent was obtained from the patients after the Research Ethics Committee approved this study.

^{18}F -FETNIM PET/CT protocol and imaging analysis

With no fasting required, all patients underwent ^{18}F -FETNIM PET/CT scans 1 to 3 days before surgery. The method of ^{18}F -FETNIM radiosynthesis was performed as previously reported by Yang et al. [14]. The tracer was prepared using a commercial available synthesizer (GE TRACERLab FXN). The radiochemical purity of the ^{18}F -FETNIM exceeded 99%, and its specific radioactivity exceeded 300 GBq/ μmol . Patients received a dose of 3.70 MBq/kg (0.1 mCi/kg) ^{18}F -FETNIM intravenously and then rested for approximately 120 min. To accurately match the region of ^{18}F -FETNIM uptake and the tissue specimen for immunohistochemical staining, the orbitomeatal line (OML) was perpendicular to the scanning table. Scanning of the head was performed with an integrated PET/CT device (Discovery LS, GE Healthcare, Milwaukee, WI). An unenhanced CT scan was performed at a voltage of 140 kV, a current of 80 mA, and a pitch of 6. Immediately after the CT scan, a PET emission scan was performed. The acquisition time for PET was 4 min per bed position. The PET datasets and CT data were reconstructed iteratively with an ordered subsets expectation maximization algorithm and segmented attenuation correction (2 iterations, 21 subsets). The attenuation-corrected PET images, CT images, and fused

PET/CT images were transferred to a Xeleris workstation (GE Healthcare, Haifa, Israel) for image analysis.

Axial, coronal, and sagittal attenuation-corrected images were qualitatively analyzed by two experienced nuclear medicine physicians blinded to the pathological diagnosis. ^{18}F -FETNIM uptake was examined as a continuous variable with semiquantitative measurement. Regions of interest (ROIs) were placed over the entire tumor, normal tissue, and ventricle in each slice of the image. A maximum area of 3×3 pixels (7.04×7.04 mm) was determined with an automated system to represent the highest concentration of radioactivity in the tumor. The standardized uptake value (SUV) was determined as follows: activity concentration/(injected dose/body weight). The maximum SUV (SUVmax) in the tumor was measured.

Sampling of tumor specimens

Because the tumors were heterogeneous to tracer uptake, the tissue specimen for the biological marker was accurately matched to the ^{18}F -FETNIM uptake region as follows. First, ^{18}F -FETNIM uptake on the PET/CT image was identified before tumor resection. Second, gross maximal safe resection was performed. After resection, the plane of the OML and the ligature between the cranial side and the caudal side were marked on the tumor specimen. Third, the plane of the OML was perpendicular to the table, from the cranial side to the caudal side, and the specimen was cut consecutively at approximately 4.5-mm intervals, which ensured that each specimen slice matched the PET/CT slice. Fourth, the tissue specimen was selected for immunohistochemical staining according to the area of ^{18}F -FETNIM uptake. All slices were fixed with 10% formalin and then embedded in paraffin. Whole-mount paraffin sections were made from these slices, cut consecutively into sets of 3–5 μm sections and stained with hematoxylin and eosin (HE). Then, the glass slides were routinely processed for HE and immunohistochemical staining.

Immunohistochemical staining and analysis

Immunohistochemical markers related to hypoxia, angiogenesis, proliferation, and invasion, such as hypoxia-inducible factor-1-alpha (HIF-1 α), Ki-67, vascular endothelial growth factor (VEGF), and matrix metalloproteinase 9 (MMP-9), were evaluated in the glioma samples. Immunohistochemical staining was performed as described previously [18]. Briefly, the sections were deparaffinized and heated to 100 $^{\circ}\text{C}$ for 5 min for antigen retrieval in ethylenediaminetetraacetic acid buffer (pH 9.0). After the sections were cooled, endogenous peroxidase quenching was performed by incubating the slides with 3% hydrogen peroxidase for 5 min at room temperature. Then, the slides were incubated for 1 h with the following primary antibodies: a mouse monoclonal antibody against

HIF-1 α (AB8366; Abcam, Tokyo, Japan; 1:1000 dilution), an antibody against Ki-67 (MIB1, Santa Cruz, Shanghai, China, 1:50 dilution), a monoclonal antibody against VEGF (Immuno-Biological Laboratories, Takasaki, Japan; 1:100 dilution), and an antibody against MMP-9 (56-2A4, Abcam, Temecula, TX, USA, 1:200 dilution). An EnVision TM + anti-rabbit HRP-labeled polymer (Dako) was used as the secondary antibody and incubated for 30 min. Then, 3,3'-diaminobenzidine tetrahydrochloride (DAB) was applied for color development at room temperature for 5 min, and sections were subsequently counterstained with hematoxylin. Tissue specimens positive for HIF-1 α , Ki-67, VEGF, and MMP-9 were used as positive controls. Negative control slides without primary antibodies were included for each stain. Five fields (400 \times) were analyzed to determine the extent of HIF-1 α - and Ki-67-stained nuclei. For HIF-1 α and Ki-67, the percentage of cells with positive nuclei was counted. For VEGF and MMP-9, the percentage of cells with positive staining in the membrane and/or focal cytoplasmic staining was counted.

Treatment and follow-up

The management of the patients was designed according to the National Comprehensive Cancer Network (NCCN) guidelines for Central Nervous System Cancers, Version 1.2011, and was performed by the attending radiation and medical oncologists after surgery. Treatment decisions did not consider the ^{18}F -FETNIM PET/CT finding. Patients with high-risk LGG and HGG received postoperative concurrent chemoradiotherapy (CCRT), which delivered 54 Gy for high-risk LGG and 60 Gy for HGG in 2.0 Gy fraction with the concomitant administration of oral temozolomide (TMZ) (75 mg/m 2), followed by adjuvant TMZ (150–200 mg/m 2 for 5/28 days) for 6 cycles. After treatment, patients were evaluated every 3–6 months during the first 5 years and every 6–12 months thereafter. OS was defined as the interval from the date of diagnosis upon study enrolment until either the date of death from any cause or the date of the last follow-up for living patients.

Statistical analysis

Statistical analyses were performed using SPSS version 22.0 statistical software (SPSS, Inc., Chicago, IL). The SUVmax and immunohistochemical variables were compared between LGG and HGG using the *T* test. The nonparametric Spearman rank test was used to analyze the correlation between ^{18}F -FETNIM uptake and the immunohistochemical variables. OS was calculated with the Kaplan-Meier method, and differences in the survival curves between the 2 groups were analyzed by the log-rank test. A multivariate analysis was performed to identify the prognostic factors influencing OS using the Cox proportional hazards regression model. All of the tests

Table 1 Patient and tumor characteristics

Patient/tumor	Number (percentages)
Age	
≤40 years	17 (68%)
>40 years	8 (32%)
Gender	
Male	16 (64%)
Female	9 (36%)
Pathological type	
II	8 (32%)
III	7 (28%)
IV	10 (40%)

were two-tailed, and $P < 0.05$ was considered statistically significant.

Results

Patient and tumor characteristics

In total, 25 patients were analyzed. Histological analysis revealed that 8 patients had high-risk grade II gliomas (5 astrocytomas, 3 oligoastrocytomas), 7 patients had grade III gliomas (3 anaplastic astrocytomas, 2 anaplastic oligodendrogliomas, and 2 anaplastic oligoastrocytomas), and 10 patients had grade IV gliomas (9 glioblastomas, 1 gliosarcoma). Patient and tumor characteristics are shown in Table Table 1.

^{18}F -FETNIM uptake in gliomas

For all of the tumors, the SUVmax ranged from 0.69 to 2.7, with a median of 1.68 (1.66 ± 0.51). The (mean \pm SD) SUVmax values in gliomas of grades II, III, and IV were 1.08 ± 0.27 , 1.70 ± 0.15 , and 2.1 ± 0.33 , respectively. The SUVmax was significantly higher in grade IV gliomas than in either grade III or II gliomas ($P = 0.007$, $P < 0.001$). The difference in SUVmax was significant between grade III and grade II gliomas ($P < 0.001$). A correlation between the SUVmax and glioma grade was found ($r = 0.881$,

$P < 0.001$). Figure 1 shows that ^{18}F -FETNIM uptake in a grade IV glioma was much greater than that in other brain tissues. Figure 2 shows that ^{18}F -FETNIM uptake in a grade III glioma was similar to that in other brain tissues.

Immunohistochemical findings

Positive HIF-1 α and Ki-67 expression was recognized as brown staining in the nucleus. The majority of grade III and IV gliomas showed moderate to strong expression, whereas the expression in LGGs was weak. Positive VEGF and MMP-9 expression was observed as brown staining mainly in the cytoplasm and/or membranes. VEGF expression was uniformly high in the majority of HGGs and moderately high in LGGs. MMP-9 expression was moderate to strong in HGGs and weak to moderate in LGGs. The levels of HIF-1 α , Ki-67, VEGF, and MMP-9 were significantly higher in HGGs than in LGGs (Table Table 2). The expression of different biological markers is shown in Fig. 3, and the descriptive statistics for all of the variables are shown in Table Table 2.

Correlation between ^{18}F -FETNIM uptake and immunohistochemical staining

The level of ^{18}F -FETNIM uptake was divided into 2 groups using the median SUVmax as the basis for the division. The expression levels of HIF-1 α , Ki-67, VEGF, and MMP-9 in gliomas with high ^{18}F -FETNIM uptake were much greater than those in gliomas with low ^{18}F -FETNIM uptake, and the differences were statistically significant. The detailed data are shown in Table Table 2. The SUVmax was significantly correlated with the expression of HIF-1 α , Ki-67, VEGF, and MMP-9 ($r = 0.820, 0.747, 0.606$, and 0.727 , respectively; all $P < 0.001$).

^{18}F -FETNIM PET/CT uptake and OS

Of all the patients, 10 were alive and 15 died, with a median follow-up time of 29.5 (3–101.6) months. The 3-year OS rate for all patients was 49.4% (Fig. 4a). Patients with a high SUVmax had significantly worse 3-year OS than those with

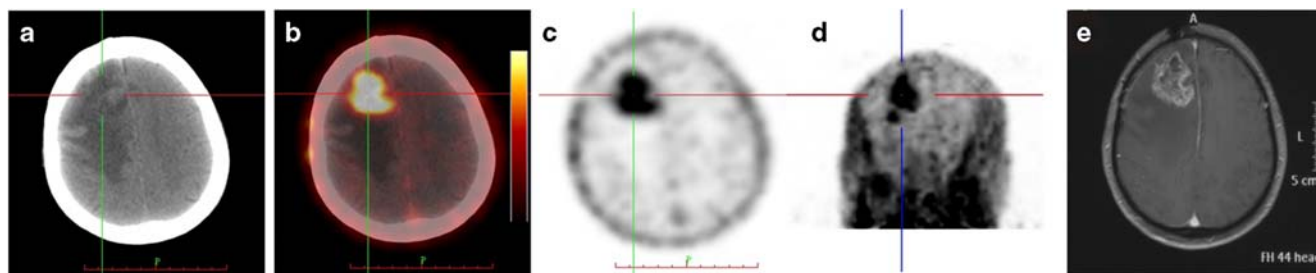


Fig. 1 Images of a grade IV glioma. ^{18}F -FETNIM uptake in tumor tissues was much greater than that in brain tissues. **a** CT image. **b** PET image. **c** Fusion transaxial PET/CT image. **d** Coronal PET image. **e** Transaxial contrast-enhanced T1-weighted MR image

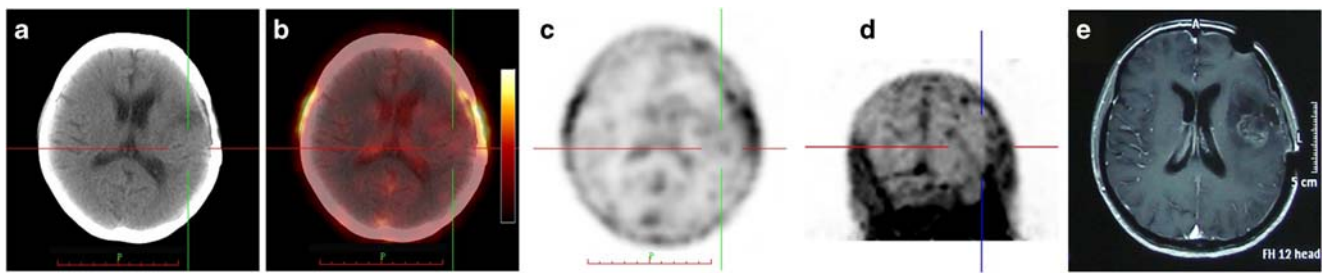


Fig. 2 Images of a grade III glioma. ^{18}F -FETNIM uptake in tumor tissue was similar to that in brain tissues. **a** CT image. **b** PET image. **c** Fusion transaxial PET/CT image. **d** Coronal PET image. **e** Transaxial contrast-enhanced T1-weighted MR image

a low SUVmax (24.4% vs. 70.3%, $P=0.003$), as shown in Fig. 4b.

A multivariate Cox proportional hazards model was constructed to evaluate age, sex, tumor grade, and SUVmax as predictors of OS. The univariate analysis showed that the SUVmax was a significant predictor of OS, and the grade tended to have predictive value. The multivariate analysis showed that no factor significantly predicted OS, as illustrated in Table 3.

Discussion

In this study, we used a new hypoxia tracer, ^{18}F -FETNIM, to detect hypoxia in glioma and obtain more biological information that can aid in increasing our understanding of malignant tumor progression.

It has been reported that ^{18}F -FETNIM PET/CT is useful as a noninvasive hypoxia imaging tool for detecting the distribution of tumor hypoxia in patients with non-small cell lung cancer [2, 15], esophageal cancer [16], and head and neck cancer [19]. In the present study, ^{18}F -FETNIM PET/CT was used to identify hypoxia in glioma. ^{18}F -FETNIM uptake was uniformly lower in overall healthy brain tissue than in glioma tissue. This finding was consistent with a previous study of ^{18}F -FETNIM biodistribution in rats with mammary carcinoma, which showed that the lowest uptake of ^{18}F -FETNIM was detected in the cerebellum [17]. This finding may be explained by the low lipophilicity and rapid clearance of this tracer from brain tissue compared with those of ^{18}F -FMISO. Therefore, ^{18}F -FETNIM is an ideal tracer for PET to image hypoxia in tumors of the central nervous system. Based on the

semiquantitative analysis, the median SUVmax was 1.68 (1.66 ± 0.51). The SUVmax in grade IV gliomas (2.1 ± 0.33) was significantly higher than that in grade III gliomas (1.70 ± 0.15), which was higher than that in grade II gliomas (1.08 ± 0.27). The uptake of ^{18}F -FETNIM was similar to that of FRP-170, which showed SUVmax values of 1.6, 2.3, and 1.9 in the 3 patients with GBM and 1.3–1.5 in the 5 patients with LGG [7]. In the present study, further analysis showed a correlation between ^{18}F -FETNIM uptake and glioma grade. This finding was consistent with previous ^{18}F -FMISO studies using different parameters. Cher et al. [8] showed that all grade IV tumors demonstrated high ^{18}F -FMISO uptake and that low grade tumors universally had little to no tracer uptake. In the study by Yamamoto et al. [9], the mean tumor-to-normal brain tissue (T/N) ratio and the maximum and mean tumor-to-blood (T/Bmax, T/Bmean) ratios in grade IV gliomas were all significantly higher than those in grade III gliomas. Kanoto et al. [10] reported a strong positive correlation between tumor grade and the maximum and mean tumor-to-normal (T/Nmax, T/Nmean) ratios. Additionally, both T/Nmax and T/Nmean could differentiate LGG from HGG. Hirata et al. [11] reported that the tumor-to-cerebellum ratio of FMISO uptake was higher in GBM than in non-GBM tumors. In this study, although we used only a simpler parameter, SUVmax, for the measurement of hypoxia in gliomas, the results indicate that ^{18}F -FETNIM PET/CT is a promising tool that can be used to detect hypoxia in gliomas.

During rapid and aberrant tumor cell proliferation, an imbalance between oxygen supply and demand and the rapid depletion of nutrients and vascular obstruction occur, which result in hypoxia in the tumor. Hypoxia further induces more aggressive tumor cells and promotes malignant progression

Table 2 The expression level of different biological markers and the difference between different disease

	Median (range)	Mean \pm SD	HGG	LGG	P^*	High uptake	Low uptake	P^*
HIF-1 α	27% (1%–74%)	28.44% \pm 20.94	39.53% \pm 15.62	4.88% \pm 3.48	<0.001	44.5% \pm 15.88	13.62% \pm 12.23	<0.001
Ki-67	36% (1%–53%)	26.99% \pm 18.32	38.47% \pm 8.10	2.60% \pm 0.98	<0.001	39.75% \pm 8.58	15.22% \pm 17.05	<0.001
VEGF	70% (20%–90%)	62.24% \pm 22.06	75.29% \pm 10.03	34.5% \pm 12.04	<0.001	75.75% \pm 10.25	49.77% \pm 22.93	=0.002
MMP-9	54% (24%–78%)	49.76% \pm 15.39	57.35% \pm 10.71	33.63% \pm 10.62	<0.001	60.08% \pm 10.67	40.23% \pm 12.83	<0.001

* T test

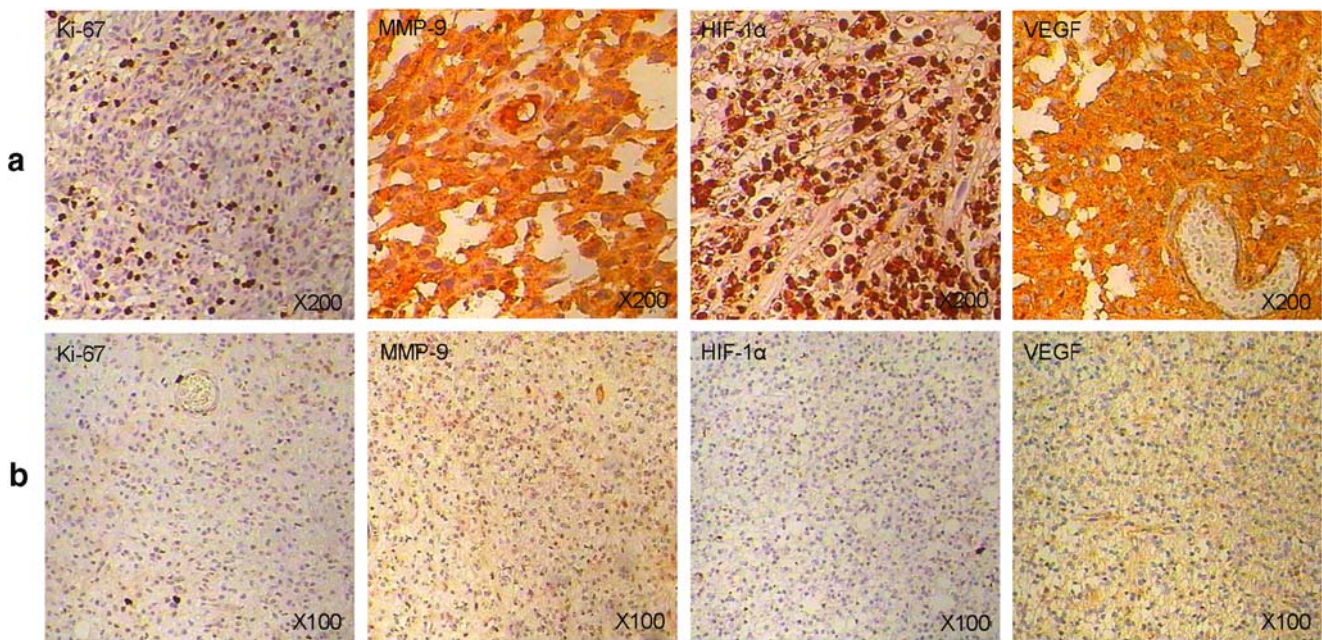


Fig. 3 Expression of different biological markers by immunohistochemical staining. **a** A row of markers (original magnification $\times 200$): immunohistochemical analysis with a high SUVmax showed high expression of Ki-67, MMP-9, HIF-1 α , and

VEGF. **b** A row of markers (original magnification $\times 100$): immunohistochemical analysis with a low SUVmax showed low expression of Ki-67, MMP-9, HIF-1 α , and VEGF

and resistance to therapy [20]. We further assessed the relationship between the uptake of ^{18}F -FETNIM and the expression of biomarkers related to angiogenesis, proliferation, and the invasion of glioma. The results showed that the SUVmax of the tumor was significantly correlated with the levels of Ki-67, MMP-9, HIF-1 α , and VEGF expression, which may imply that higher ^{18}F -FETNIM uptake can predict tumors with more aggressive behaviors and malignant progression. It is noteworthy that the whole tumor was completely surgically resected via craniotomy, and the tissue specimen obtained for immunohistochemical staining accurately matched the region of ^{18}F -FETNIM uptake in the present study. In previous studies, limited data revealed a correlation between hypoxia, as detected by PET or PET/CT, and angiogenesis, proliferation,

and invasion at the protein level, as determined by immunohistochemistry (IHC) in gliomas. The results were inconsistent because the specimens were obtained with different methods. In the study by Kawai et al. [21], the specimens were collected using a navigation system to identify the precise region concerning the ^{18}F -FMISO PET image in HGG. The results showed that ^{18}F -FMISO uptake was significantly correlated with the expression of VEGF. However, it was not correlated with HIF-1 α expression, which might be because HIF-1 α expression was not significantly different between grade III and grade IV gliomas. Two early studies did not show a correlation between ^{18}F -FMISO uptake and the expression of HIF1 α , VEGF, or Ki-67 in glioblastoma [8, 22]. The specimens were obtained regardless of the region of

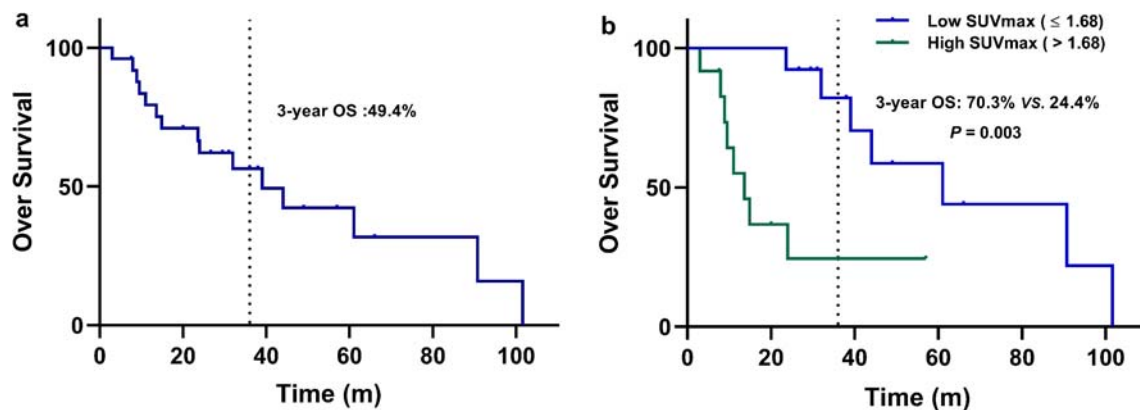


Fig. 4 Kaplan-Meier survival analysis in glioma patients. **a** OS in all patients. **b** The 3-year OS time in patients with a low SUVmax was significantly longer than that in those with a high SUVmax. OS, overall survival; LGG, low grade glioma; HGG, high grade glioma

hypoxia tracer distribution in these two studies. Our findings indicate that ^{18}F -FETNIM PET/CT can provide information on malignant progression in a noninvasive manner.

It is well known that hypoxia is associated with poor OS. The results of this study showed that patients with a higher SUVmax or a more advanced grade have worse prognoses than those with a lower SUVmax. However, the multivariate analysis showed that neither grade nor SUVmax was an independent prognostic factor for OS. Previous studies of ^{18}F -FMISO PET/CT for predicting OS in glioma have yielded inconsistent results due to relatively small sample sizes and the inclusion of heterogeneous tumors. Spence et al. [22] reported that survival was shorter in 22 glioblastoma multiforme patients who had maximum tissue to blood concentration value (T/Bmax) ratios. The multivariate analyses found that the T/Bmax ratio and hypoxic volume (HV) were strongly associated with survival compared with age and the Karnofsky Performance Status (KPS). Bekaert et al. [12] showed significantly shorter OS in the ^{18}F -FMISO uptake group than in the no uptake group in a cohort of 33 glioma patients. However, the multivariate Cox analysis indicated that ^{18}F -FMISO uptake, grade, extent of resection, age, or performance status (PS) did not significantly predict OS. The SUVmax on ^{18}F -FETNIM PET-CT and tumor grade were both predictive for patient survival based on the univariate analysis. Therefore, we performed a multivariate analysis. Unfortunately, there was no significant independent predictor of OS on the multivariate analysis, including either SUVmax or tumor grade, the latter of which is a known predictor. This may result from either the small sample size of our cohort or the interplay between the SUVmax and tumor grade. Nonetheless, there was a trend that the SUVmax was a strong predictor of OS. We will further explore more samples and confirm independent predictors. Kawai et al. [21] revealed that OS was significantly shorter in patients with a higher T/Bmax ratio than in those with lower uptake in a cohort of 32 patients, but multivariate survival analyses were not performed. At least two limitations of this study need to be mentioned. First, the size of our study population was relatively small, which may cause

statistical bias. Second, the only ^{18}F -FETNIM PET uptake parameter used for analysis was the SUVmax. Therefore, future studies with large samples are needed to confirm the clinical value of hypoxic PET/CT imaging.

In conclusion, ^{18}F -FETNIM PET/CT provides an excellent noninvasive assessment of hypoxia in glioma. High ^{18}F -FETNIM uptake can provide important information about tumor hypoxia, angiogenesis, proliferation, invasion, and the grade of glioma. The SUVmax on ^{18}F -FETNIM PET/CT was the strongest significant predictor of OS in the univariate analysis and the strongest, albeit nonsignificant, predictor of OS in the multivariate analysis. ^{18}F -FETNIM PET/CT might become useful in evaluating novel therapeutic agents that target tumor hypoxia in clinical trials if the results of the present study are replicated and confirmed in larger prospective studies.

Funding information This study was funded by the Science Technology Program of Jinan (201805051) and the Key Research Development Program of Shandong Province (2019GGX101057).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Ries LAG, Eisner MP, Kosary CL, et al. SEER cancer statistics review, 1975–2001. National Cancer Institute; Bethesda: 2004. [Accessed July 26, 2018].
2. Hu M, Xing L, Mu D, et al. Hypoxia imaging with ^{18}F -fluoroerythronitroimidazole integrated positron emission tomography and computed tomography and immunohistochemical studies in non-small-cell lung cancer. *Clin Nucl Med.* 2013;38:591–6.
3. Chatterjee A, Gupta T, Rangarajan V, et al. Optimal timing of fluorine-18-fluoromisonidazole positron emission tomography/computed tomography for assessment of tumor hypoxia in patients with head and neck squamous cell carcinoma. *Nucl Med Commun.* 2018;39:859–64.
4. Mapelli P, Zerbetto F, Incerti E, et al. ^{18}F -FAZA PET/CT hypoxia imaging of high-grade glioma before and after radiotherapy. *Clin Nucl Med.* 2017;42:e525–6.
5. Zegers CM, Hoebbers FJ, van Elmpt W, et al. Evaluation of tumour hypoxia during radiotherapy using [^{18}F]HX4 PET imaging and blood biomarkers in patients with head and neck cancer. *Eur J Nucl Med Mol Imaging.* 2016;43:2139–46.

Table 3 Univariate and multivariate analysis of prognostic factors in OS for glioma patients

	Univariate analysis			Multivariate analysis		
	<i>P</i> *	HR	95.0% CI	<i>P</i> *	HR	95.0% CI
Gender	0.404	1.743	0.472–6.439	0.734	0.738	0.128–4.266
Age	0.253	2.192	0.571–8.416	0.575	1.675	0.276–10.163
Grade	0.052	4.441	0.989–19.942	0.296	2.487	0.451–13.726
SUVmax	0.007	5.593	1.605–19.492	0.108	3.470	0.762–15.799

*Log-rank test

HR, hazard ratio; CI, confidence interval

6. Lopci E, Grassi I, Rubello D, et al. Prognostic evaluation of disease outcome in solid tumors investigated with ^{64}Cu -ATSM PET/CT. *Clin Nucl Med*. 2016;41:e87–92.
7. Shibahara I, Kumabe T, Kanamori M, et al. Imaging of hypoxic lesions in patients with gliomas by using positron emission tomography with 1-(2-[^{18}F] fluoro-1-[hydroxymethyl]ethoxy)methyl-2-nitroimidazole, a new ^{18}F -labeled 2-nitroimidazole analog. *J Neurosurg*. 2010;113(2):358–68.
8. Cher LM, Murone C, Lawrentschuk N, et al. Correlation of hypoxic cell fraction and angiogenesis with glucose metabolic rate in gliomas using ^{18}F -fluoromisonidazole, ^{18}F -FDG PET, and immunohistochemical studies. *J Nucl Med*. 2006;47:410–08.
9. Yamamoto Y, Maeda Y, Kawai N, et al. Hypoxia assessed by ^{18}F -fluoromisonidazole positron emission tomography in newly diagnosed gliomas. *Nucl Med Commun*. 2012;33:621–5.
10. Kanoto M, Kirii K, Hiraka T, et al. Correlation between hypoxic area in primary brain tumors and WHO grade: differentiation from malignancy using ^{18}F -fluoromisonidazole positron emission tomography. *Acta Radiol*. 2018;59:229–35.
11. Hirata K, Terasaka S, Shiga T, et al. ^{18}F -Fluoromisonidazole positron emission tomography may differentiate glioblastoma multiforme from less malignant gliomas. *Eur J Nucl Med Mol Imaging*. 2012;39:760–70.
12. Bekaert L, Valable S, Lechapt-Zalcman E, et al. [^{18}F]-FMISO PET study of hypoxia in gliomas before surgery: correlation with molecular markers of hypoxia and angiogenesis. *Eur J Nucl Med Mol Imaging*. 2017;44:1383–92.
13. Grönroos T, Bentzen L, Marjamäki P, et al. Comparison of the biodistribution of two hypoxia markers [^{18}F]FETNIM and [^{18}F]FMISO in an experimental mammary carcinoma. *Eur J Nucl Med Mol Imaging*. 2004;31:513–20.
14. Yang DJ, Wallace S, Cherif A, et al. Development of F-18-labeled fluoroerythronitroimidazole as a PET agent for imaging tumor hypoxia. *Radiology*. 1995;194:795–800.
15. Li L, Hu M, Zhu H, et al. Comparison of ^{18}F -fluoroerythronitroimidazole and ^{18}F -fluorodeoxyglucose positron emission tomography and prognostic value in locally advanced non-small-cell lung cancer. *Clin Lung Cancer*. 2010;11:335–40.
16. Yue J, Yang Y, Cabrera AR, et al. Measuring tumor hypoxia with ^{18}F -FETNIM PET in esophageal squamous cell carcinoma: a pilot clinical study. *Dis Esophagus*. 2012;25:54–61.
17. Grönroos T, Eskola O, Lehtiö K, et al. Pharmacokinetics of [^{18}F]FETNIM: a potential marker for PET. *J Nucl Med*. 2001;42:1397–13404.
18. Xue S, Hu M, Li P, et al. Relationship between expression of PD-L1 and tumor angiogenesis, proliferation, and invasion in glioma. *Oncotarget*. 2017;8:49702–12.
19. Lehtio K, Oikonen V, Nyman S, et al. Quantifying tumour hypoxia with fluorine-18 fluoroerythronitroimidazole ([^{18}F]FETNIM) and PET using the tumour to plasma ratio. *Eur J Nucl Med Mol Imaging*. 2003;30:101–8.
20. Kaur B, Khwaja FW, Severson EA, et al. Hypoxia and the hypoxia-inducible-factor pathway in glioma growth and angiogenesis. *Neuro-Oncology*. 2005;7:134–53.
21. Kawai N, Lin W, Cao WD, et al. Correlation between ^{18}F -fluoromisonidazole PET and expression of HIF-1 α and VEGF in newly diagnosed and recurrent malignant gliomas. *Eur J Nucl Med Mol Imaging*. 2014;41:1870–8.
22. Spence AM, Muzi M, Swanson KR, et al. Regional hypoxia in glioblastoma multiforme quantified with [^{18}F]fluoromisonidazole positron emission tomography before radiotherapy: correlation with time to progression and survival. *Clin Cancer Res*. 2008;14:2623–30.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.