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Prognostic Significance of Dynamic ^{18}F-FET-PET in Newly Diagnosed Astrocytic High Grade Glioma

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ABSTRACT

Despite advances in diagnosis and use of different therapeutic regimens in astrocytic high grade glioma (HGG), the prognosis for patients remains grim. Additional pre-therapeutic information is needed to tailor the management. In order to gain additional prognostic information at primary diagnosis we investigated the value of dynamic ^{18}F-fluoro-ethyl-tyrosine (^{18}F-FET)-PET.

Methods: 121 patients with primary diagnosis of an astrocytic HGG (51 WHO III°; 70 WHO IV°) and dynamic ^{18}F-FET-PET investigation prior to histopathological assessment were retrospectively evaluated. ^{18}F-FET-PET analysis comprised the assessment of static parameters (maximal and mean tumor uptake (SUV_{max}/BG and SUV_{mean}/BG), biological tumor volume (BTV)), as well as dynamic time-activity-curves including the evaluation of minimal time-to-peak (TTP_{min}). The prognostic influence of PET parameters as well as other clinical parameters on progression free and overall survival (PFS and OS) was evaluated using uni- and multivariate Cox regression and Kaplan-Meier survival estimates.

Results: In the overall group, median PFS and OS were 12 and 22 months. SUV_{max}/BG, SUV_{mean}/BG and BTV were significantly higher in WHO IV° compared to WHO III° gliomas, median TTP_{min} was 12.5 minutes in both groups. Univariately, the factors age, WHO grade, O6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation status, contrast enhancement, initial treatment and TTP_{min} showed prognostic significance, with WHO grade, *MGMT* status, age and TTP_{min} remaining significant in the multivariate analysis. WHO grade and TTP_{min} reached a similar fit for the prognostic

evaluation. The prognosis of WHO III° astrocytoma with early TTP_{min}≤12.5 did not differ significantly from GBM patients.

Conclusions: Early TTP_{min} is associated with worse outcome in patients with newly diagnosed astrocytic HGG. In the preoperative setting, TTP_{min} can be a valuable non-invasive prognostic marker with comparable significance to WHO grade. Additionally, TTP_{min} can help identify highly aggressive tumors among WHO III° astrocytoma and might help adjusting standard treatment towards an individualized, risk-adapted therapy regime.

Keywords: High grade glioma, ^{18}F-FET-PET, kinetic analysis, prognostic value

INTRODUCTION

Management of astrocytic high grade glioma has changed during the last ten years. The introduction of radiotherapy plus concomitant and adjuvant temozolomide, for example, has significantly improved the prognosis of patients with glioblastoma (GBM; World Health Organization (WHO) IV°) (*1,2*); for WHO III° astrocytoma, the therapeutic management is more variable and chosen individually (*3-7*). Current treatment recommendations vary from resection or biopsy followed by radio- or chemotherapy or combined modality treatment (*8*). In consideration of the variable disease course in WHO III° astrocytoma patients, with some patients presenting with rapidly progressing clinical course which does not differ significantly from GBM patients, the identification of prognostic markers would be most helpful for treatment stratification.

In the last years, O6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation and mutations in the cytosolic isocitrate dehydrogenase (IDH) 1 or 2 gene have been identified as molecular-genetic factors with favourable prognosis in astrocytic HGG, while loss of heterozygosity on chromosomes 1p and 19q (LOH 1p/19q) is highly correlated with an oligodendroglial morphology (*9-11*). Beside molecular-genetic biomarker profiles, which can be assessed invasively only, molecular imaging including positron emission tomography (PET) with radiolabelled amino acids or their analogues are increasingly used for tumor characterisation and prognostic evaluation (*12-14*); their potential as imaging derived, non-invasive biomarker is currently under investigation.

One of the most promising radiotracers is the ^{18}F-labelled fluoroethyltyrosine (^{18}F-FET), which shows high uptake in glioma cells and low uptake in healthy brain tissue, resulting in an excellent tumor-to-background-contrast (*15*). In this context, especially *dynamic*

acquisition of ^{18}F-FET-PET has gained increasing interest, as it enables glioma characterization by differentiating between low and high grade glioma (LGG; HGG) tissue by analysis of individual time-activity-curves of ^{18}F-FET-FET-uptake (*16-19*). These time-activity-curves are typically increasing in LGG and decreasing in HGG. However, decreasing time-activity-curves were recently also observed in histologically verified LGG, indicating a higher risk for poor outcome (*20*). Another study has reported a characteristic change of time-activity-curves during malignant transformation of low grade to high grade astrocytoma (*21*).

In the current retrospective study, we aimed to identify dynamic ^{18}F-FET-PET parameters associated with tumor aggressiveness in patients with newly diagnosed, histologically confirmed astrocytic HGG. We focused on time-activity-curve patterns and more specifically on the prognostic impact of the time to maximal ^{18}F-FET-uptake.

MATERIALS AND METHODS

Patient Evaluation

Patients with a newly diagnosed supratentorial astrocytic glioma WHO III° or IV° who had received dynamic ^{18}F-FET-PET prior to histological diagnosis at the Ludwig-Maximilians-University of Munich, Germany, between 2004 and 2012 were retrospectively identified. Patients with an oligoastrocytoma or oligodendroglioma were excluded to ensure homogeneity of the patient collective. The study was approved by the institutional review board and all subjects signed a written informed consent. Primary end point of the study was overall survival (OS), secondary end point progression-free survival (PFS). PFS was calculated from the baseline PET-scan to the first event of

clinical deterioration, i.e. new neurological symptoms, worsening as indicated by Karnofsky-Perfomance-Score (KPS) or an increase in administered steroid medication, or tumor growth on conventional MRI. OS was correspondingly measured from baseline PET-scan to date of death. Date of last follow-up was November 2013.

^{18}F-FET-PET And MR Imaging

Dynamic ^{18}F-FET-PET-scans were acquired with an ECAT EXACT HR+ scanner (Siemens Healthcare) according to standard protocols (*20*) after slow intravenous bolus injection of 180 MBq ^{18}F-FET. Dynamic emission recording in 3D-mode consisting of 16 frames (7x10 s, 3x30 s, 1x2 min, 3x5 min, and 2x10 min) was conducted until 40 minutes post injection. For further evaluation, images were transferred to a HERMES work station (Hermes Medical Solutions, Sweden).

Semi-quantitative evaluation included the maximal tumoral ^{18}F-FET-uptake corrected for mean background activity in the contralateral hemisphere (SUV_{max}/BG), and an estimated biological tumor volume (BTV) defined by semi-automatic threshold-based calculation of a volume of interest (SUV/BG≥1.8). Furthermore, the mean tracer uptake within the BTV (SUV_{mean}/BG) was assessed.

For dynamic evaluation of ^{18}F-FET-uptake, a 90% iso-contour threshold region of interest was defined on the 10-30 minutes summation images (frames 13-15) on each individual slice throughout the tumo,r and applied to the dynamic PET data in order to extract the time-activity-curves. Due to low count rates and the risk of noise artefacts in the very early time frames, only frames 11-16 (3-40 minutes) were considered for further analysis. For each slice within the tumor, the frame with the peak uptake was identified. The starting time of the frame plus half of the frame duration, corresponding to the

respective peak value, was set as time to peak (TTP). Accordingly, TTP accounted for 4 minutes in frame 11, 7.5 minutes in frame 12, 12.5 minutes in frame 13, 17.5 minutes in frame 14, 25 minutes in frame 15 and 35 minutes in frame 16 (see Fig. 1 for an example). The shortest TTP being present in at least two adjacent slices was defined as minimal TTP (TTP_{min}).

MR imaging was performed prior to tissue sampling according to standard protocols (*19*), which included acquisition of axial T2-weighted sequences and 3D T1-weighted sequences before and after administration of intravenous contrast agent (0.1 mmol/kg gadobenatedimeglumine (MultiHance, BraccoImaging, Milan, Italy)). Presence of contrast enhancement was evaluated as factor for further analyses.

Stereotactic Biopsies, Microsurgical Resections And Histological Evaluation

Tissue specimens for histological and molecular-genetic evaluation were obtained by either serial stereotactic biopsy or resection. Microsurgical resections were performed by experienced neurosurgeons using neuronavigation with MRI and PET image fusion (BrainLab, Heimstetten, Germany). Molecular stereotactic biopsy procedures were performed as published in detail before (*19,22*). Biopsy with an average tissue volume of approximately $1mm^3$ was obtained in the area of the highest ^{18}F-FET uptake. Mean number of obtained tumor samples per patient was five. Histological classification and tumor grading was performed according to the current WHO guidelines at the respective time point of histopathological assessment (*23*). The German Brain Tumor Reference in Bonn was consulted for complex cases. Determination of *MGMT* promoter methylation was performed using methylation-specific PCR according to standard protocols (*24*).

Statistical Analysis

SPSS for Windows (SPSS, Version 21.0, Chicago, IL) was used for statistical calculations. Length of OS and PFS was analyzed with the Kaplan-Meier-method. The distribution of patient- and tumor-related variables was analyzed by chi-squared statistics (for categorical variables) and Mann-Whitney-U test (for continuously scaled variables). Continuous parameters were reported as mean±standard deviation and range. The median was used as threshold for dichotomization of parameters. For univariate prognostic analyses, all parameters were evaluated using Cox-regression. Covariates being significant in one-variable models were then evaluated in multivariate analyses using a stepwise backwards exclusion model. In case of an inter-correlation of most relevant covariates alternative models were tested and were compared by computing the maximized likelihoods. A two-tailed p-value<0.05 was considered significant.

RESULTS

121 patients (73 males; mean age 54.0±13.7 years) with primary diagnosis of a astrocytic HGG were included (51 astrocytomas WHO III°, 70 GBM WHO IV°). The median KPS was 90 (range 60-90; only one patient with a KPS of 60). 88 patients underwent stereotactic biopsy and 33 patients microsurgical resection. Detailed results of clinical parameters including treatment details are listed in Table 1.

In the ^{18}F-FET-PET-scan, 116/121 HGG (>95%) showed enhanced ^{18}F-FET-uptake. 5 WHO III° astrocytomas, however, were found to be ^{18}F-FET-negative. SUV_{max}/BG and SUV_{mean}/BG values were higher in GBM than in WHO III° tumors (3.7 vs. 3.0; p<0.01 and 2.3 vs 2.1; p<0.01), and GBM exhibited larger BTVs (25.5 ml vs. 13.1 ml; p<0.01).

Kinetic analysis was available in 111/116 ^{18}F-FET-positive patients (43 WHO III°, 68 WHO IV°), in 10 patients kinetic analysis was not available (5 due to technical reasons, 5 due to absent ^{18}FET-uptake). Median TTP_{min} in both WHO groups was 12.5 minutes. In the WHO III° group, 22/43 (51%) patients had a TTP_{min}≤12.5 minutes (including 10 patients without contrast enhancement on MRI), in the GBM group 50/68 (74%) patients had a TTP_{min}≤12.5 minutes (see Table 1). TTP_{min} was intercorrelated with the WHO grade (p=0.02).

During the follow-up (median follow-up of survivors 61.3 months, range 7.2-101.9 months), 98/121 patients experienced tumor progression (43 WHO III°; 63 WHO IV°) and 88/121 patients had died (32 WHO III°; 62 WHO IV°).

Median PFS and OS for the entire study group was 12.2 and 21.9 months. Patients with WHO III° tumors exhibited both longer PFS and OS than GBM patients (46.2 vs. 14.2 months and 19.3 vs. 10.3 months; p≤0.001).

Univariately, WHO III°, methylated *MGMT* promoter, young age, absence of contrast enhancement, and chemo- or radiotherapy alone were associated with longer PFS and OS (see Table 2). Among the ^{18}F-FET-PET parameters, smaller SUV_{max}/BG and BTV, and late TTP_{min} were related to longer PFS. For OS, TTP_{min} correlated significantly with length of survival.

Multivariately, *MGMT* promoter methylation (hazard ratio 0.51 (95%-CI 0.33-0.81); p=0.003), TTP_{min}>12.5 minutes (hazard ratio 0.61 (95%-CI 0.38-0.98); p=0.02) were associated with longer PFS. After stepwise exclusion, the factors younger age, *MGMT* promoter methylation and TTP_{min}>12.5 minutes remained favorable predictors for OS,

while WHO grade, which was intercorrelated with TTP_{min}, was omitted in the last step. In two alternative models with inclusion of either WHO grade or TTP_{min} both models reached a similar good fit. WHO grade and TTP_{min} were both powerful predictors for OS (hazard ratio for TTP_{min}≤12.5 minutes vs. >12.5 minutes: 2.04 (95%-CI 1.20-3.48), p=0.009; for WHO IV° vs. III°: 1.73 (95%-CI 1.02-2.93), p=0.042). In the overall group, patients with a TTP_{min}>12.5 minutes had an OS of 31.8 months as compared to 15.6 months for those exhibiting a TTP_{min} ≤12.5 minutes (p=0.001; see Fig. 2). Detailed results are shown in Table 3.

Patients with a WHO III° histology and early TTP_{min}≤12.5 minutes did not have a significantly different outcome from GBM patients (PFS: 9.3 vs. 10.3 months, p=0.92; OS: 21.4 vs. 14.2 months, p=0.30), whereas those with a late TTP_{min}>12.5 minutes had favourable outcome scores (PFS: 37.4 months; median OS not reached, see Figure 3). Both WHO III° groups exhibiting either early or late TTP_{min} did not differ in terms of the respective sample size, frequency of enhanced tumors, neurosurgical procedure (biopsy vs. surgery), *MGMT* promoter methylation status, applied treatment strategies, and all other PET-parameters (SUV_{max}/BG, SUV_{mean}/BG and BTV). The results were similar in the subgroup of non-contrast enhancing WHO III° gliomas where patients with an early TTP_{min}≤12.5 minutes (10 patients) presented with unfavorable outcome compared to those with late TTP_{min}>12.5 minutes (9 patients; PFS: 9.8 vs. 37.4 months, p<0.001; OS: 20.2 months vs. median not reached, p<0.001).

For the subgroup of WHO IV° tumors, increased TTP_{min} was also related to longer survival, however, this finding was less pronounced than in WHO III° tumors (p=0.08).

DISCUSSION

During the last years, PET using the amino acid analogue ^{18}F-FET has gained increasing interest for glioma imaging, especially the acquisition of *dynamic* FET-PET. The analysis of the kinetics of tumoral ^{18}F-FET-uptake by evaluating individual time-activity-curves has been primarily investigated in the context of tumor grading and was shown to differentiate between low and high grade glioma (*16-19*). Furthermore, time-activity-curves were shown to be helpful for the identification of an oligodendroglial tumor component in low grade tumors (*25*). Recently, first studies have reported an additional prognostic value of time-activity-curves in LGG (*20*) as well as in gliobastomas (*26*). Here, the qualitative classification into tumors with increasing versus decreasing time-activity-curves revealed an association between uptake kinetics and clinical course, with significantly better outcome of patients with increasing time-activity-curves within one WHO group. This is the first study investigating the prognostic value of dynamic ^{18}F-FET-PET in newly diagnosed, histologically verified HGG, focusing on the quantitative analysis of time-activity-curves by evaluating the TTP.

Patients' survival of the current report was in line with more recently published studies (e.g. EORTC 26981-22981-NCIC CE3 study (*1*)). Our analysis further confirmed well known prognostic factors such as *MGMT* promoter methylation status, patient's age and the presence of contrast enhancement in the initial MRI (*2,27-29*).

Our key finding was that TTP_{min} was the only factor among the analyzed ^{18}F-FET-PET parameters that gained powerful prognostic impact on PFS and OS after adjustment for the effects of all other variables. Taken into account that an intercorrelation was seen

between WHO grade and $TTP_{min,}$ we tested two alternative prognostic models, including either WHO grade or TTP_{min}. We could demonstrate that TTP_{min} was a similar good predictor of outcome as WHO grade. To which extent an imbalanced distribution of TTP_{min} values among GBM patients (i.e. a relatively small number of tumors with a long TTP_{min}) might have contributed to the less pronounced impact of TTP_{min} as compared to WHO III° tumors, remains unclear and deserves further evaluation.

Among the WHO III° patients, those with an early TTP_{min}≤12.5 minutes had a prognosis which did not significantly differ from that of GBM patients. Outcome for those exhibiting a late TTP_{min} was favorable and remarkably different from that of the poor prognosis group. It is important to note that both WHO III° glioma subgroups, splitted by dichotomized TTP_{min}, were homogeneous with regard to all analyzed factors. Moreover, the prognostic impact of TTP_{min} was preserved even for the subgroup of non-enhancing WHO III° gliomas only. Apparently, the quantitative analysis of TTP_{min} allows non-invasive identification of patients with WHO III° tumors being at particular risk for early tumor progression and death. This finding can be used for patient stratification and evaluation of risk adapted treatment strategies for WHO III° tumors in the future.

The prognostic significance of TTP_{min} fits certainly to the results of a recently published study, where the shape of time-activity-curves (qualitative comparison of increasing time-activity-curves, representing a TTP_{min}≥35 minutes, versus decreasing time-activity-curves, characterized by a TTP_{min}<35 minutes) predicted patients outcome in newly diagnosed low grade astrocytoma (*20*). Furthermore, the role of TTP was already described in another study, investigating the course of kinetic ^{18}F-FET-uptake behavior

in LGG, where progressively shorter TTP was reported during malignant transformation (*21*). Therefore, one might hypothesize that TTP reflects in some way the tumor aggressiveness, which, in contrast, cannot be represented by conventional ^{18}F-FET-PET parameters such as SUV_{max}/BG and BTV. The pathophysiological mechanisms being reflected by TTP remain unclear, as the responsible pathway for the different uptake behavior is still not fully clarified. However, as discussed in previous studies (*16,18,25*), one can speculate that following factors play an important role for the uptake dynamics: the expression of the L-aminoacid transporter type 2, which promotes the bi-directional transport through the cell membrane (*30,31*), hence leading to a higher and faster ^{18}F-FET-uptake with increasing transporter density, as well as the relative cerebral blood flow and tumor perfusion, influencing the tracer delivery (and consequently the amount and speed of tumoral tracer uptake) as well as subsequent washout after intracellular accumulation of the unbound amino acid analagon, which is not incorporated into proteins and might therefore not be trapped intracellularly (*15*). An earlier TTP_{min} might therefore reflect a higher aminoacid transporter expression and/or higher tumor perfusion, which would be in line with the results of other studies reporting a prognostic significance of tumor perfusion (*32-34*). Although a recent study claimed that the descending part of the time-activity-curves of ^{18}F-FET-uptake and perfusion parameters in perfusion weighted MRI do not correlate, they reported a high correlation with the slope of the increasing part of the time-activity-curves (*35*), indicating a certain influence of tumor perfusion at least to some extent.

Limitations of the study arise from the retrospective study design and from the heterogeneous treatment management of HGG patients, which, however, is due to the

lack of a single standardized therapy in newly diagnosed anaplastic astrocytomas. Furthermore, a relatively low number of patients with tumor resection was present in our study, which is based on the management strategy of our neurosurgical center with a highly sophisticated stereotactic biopsy unit. However, we would like to stress that no differences concerning the neurosurgical procedure (biopsy vs. surgery) or initial treatment were found in the subgroups of WHO III° gliomas with early and late TTP_{min}, so that the comparison of these subgroups seems reliable despite the non-standardized treatment. Furthermore, population homogeneity regarding the prognostic/predictive factor IDH 1/2 mutation was not assessed but would be of high interest in our group of astrocytic HGG and should therefore be investigated in future studies. The lack of information concerning the 1p/19q status can be considered as less relevant being predominantly associated to oligodendroglial tumors. No central pathology review was performed, but the German Brain Tumor Reference Center in Bonn was consulted for complex cases. Another commonly discussed limiting factor is the histopathological analysis: most tissue samples were obtained by stereotactic biopsy, which is often considered to be vulnerable to misdiagnosis (*36*); as our biopsy planning is PET-based, the probability of missing oligodendroglial tissue with usually high FET-avidity is low, yet, although it includes *dynamic* ^{18}F-FET-PET data, which differentiates between low and high grade tumor (*19*), a misdiagnosis among HGG cannot be excluded with certainty.

Our study results are promising and need to be confirmed in prospective clinical trials. As the mechanisms underlying ^{18}F-FET-kinetics are not well understood and tumor perfusion could play a role, future studies need to assess the complementary,

independent or equivalent information obtained from dynamic ^{18}F-FET-PET and perfusion MRI data for prognostic evaluation of high-grade glioma.

CONCLUSION

While static ^{18}F-FET-PET parameters do not have a prognostic significance in patients with a newly diagnosed astrocytic HGG, TTP_{min} is an independent prognostic factor with comparable significance to WHO grade: HGG with an early TTP_{min} have a significantly worse outcome than those with later TTP_{min}. The quantitative analysis of TTP_{min} allows non-invasive identification of WHO III° glioma patients being at particular risk for early tumor progression and death. This finding might be used for patient stratification and individualized risk adapted treatment strategies for astrocytic WHO III° tumors in the future.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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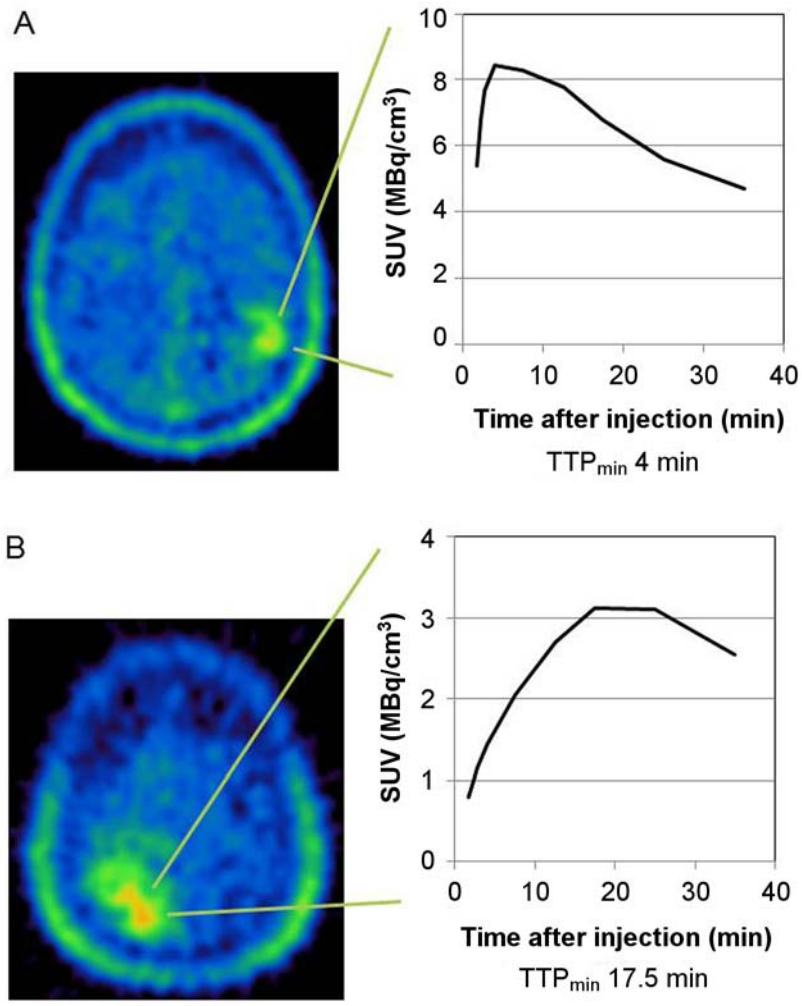

Figure 1 Time-activity-curves of ^{18}F-FET-uptake were evaluated slice by slice throughout the entire tumor in order to assess the shortest time-to-peak (TTP_{min}). A) shows an example of decreasing curve with an early peak after 4 minutes, while B) shows a late peak after 17.5 minutes.

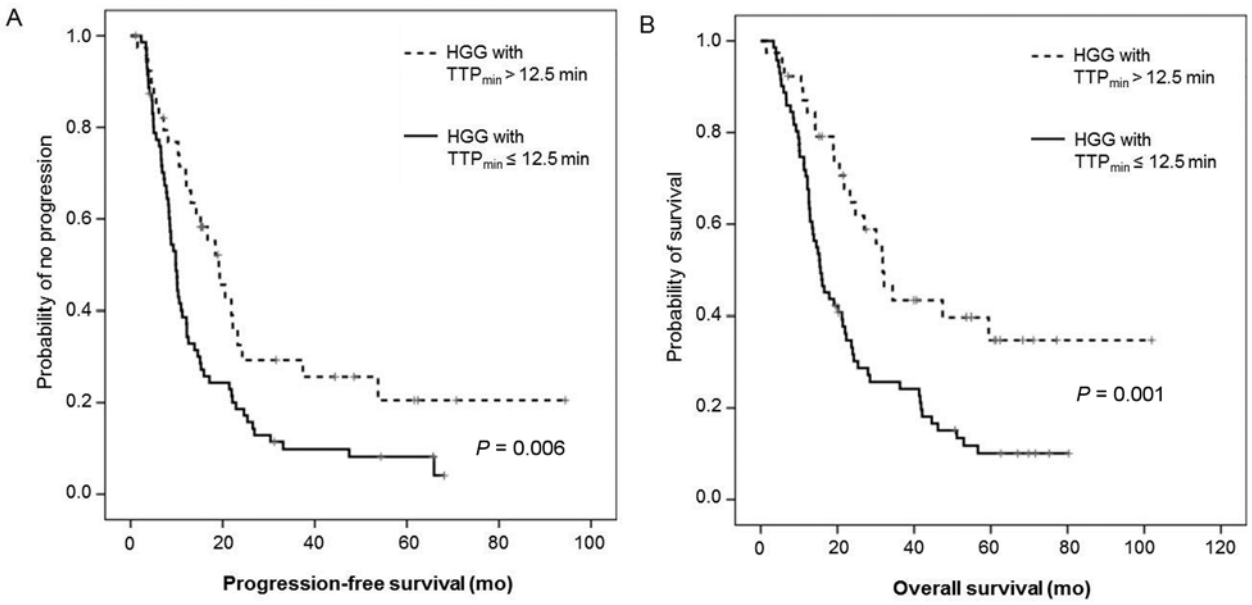

Figure 2 Kaplan-Meier estimates for PFS (A) and OS (B) of patients with newly diagnosed astrocytic HGG: plots show a significantly longer PFS and OS of patients with late TTP_{min}>12.5 minutes compared to short TTP_{min}≤12.5 minutes (p=0.006 for PFS and p=0.001 OS).

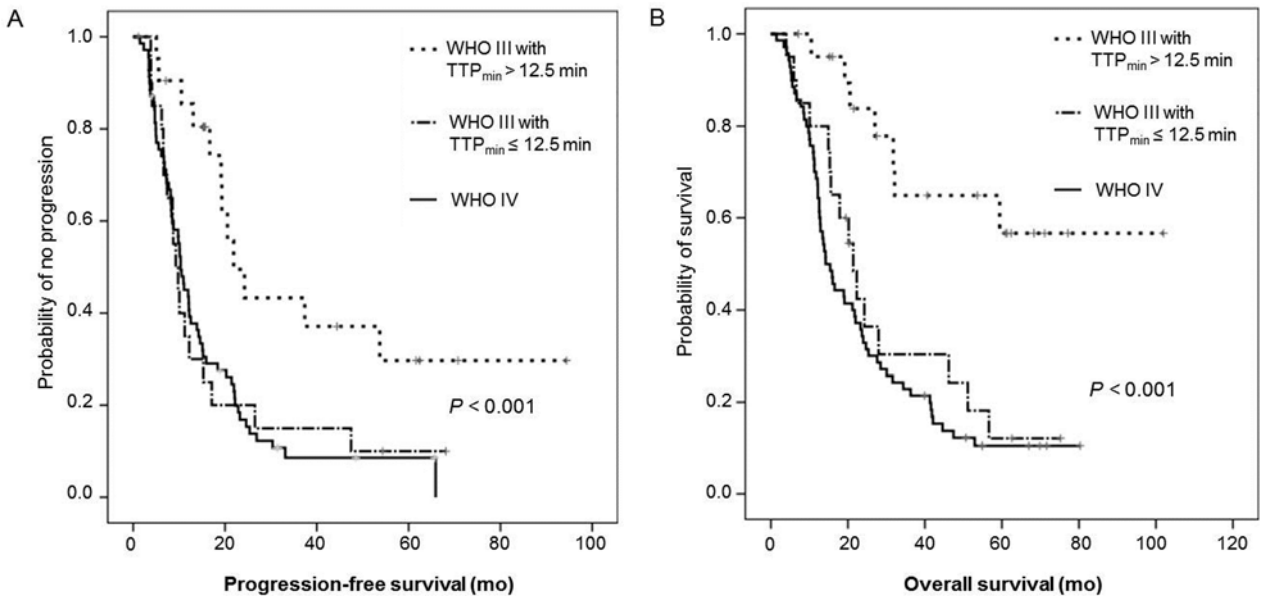

Figure 3 Kaplan-Meier estimates for PFS (A) and OS (B) of patients with newly diagnosed astrocytic HGG show a significantly longer PFS and OS of WHO III° astrocytoma patients with late TTP_{min}>12.5 minutes (p<0.001 for PFS and OS), while those with short TTP_{min}≤12.5 minutes have a comparable outcome to GBM patients (p=0.92 for PFS and p=0.30 for OS).

Table 1 Overview of clinical and FET-PET parameters according to the WHO group

	All HGG	WHO III°	WHO IV°	*p-value*
Patients (n)	121	51	70	n. s.
Age (years)	54.0	46.9	59.1	≤0.001
Sex				
male (n)	73	32	41	n. s.
female (n)	48	19	29	
MGMT				
Methylated (n)	66	33	33	0.022
Unmethylated (n)	43	12	31	
not available (n)	12	6	6	
Contrast enhancement on MRI				
yes (n)	95	25	70	≤0.001
no (n)	26	26	0	
Surgical resection (n)	33	6	27	≤0.001
Radiochemotherapy (n)	82	17	65	≤0.001
Chemotherapy (n)	23	21	2	≤0.001
Radiotherapy (n)	13	11	2	≤0.001
Brachytherapy (n)	2	2	0	n. s.
Palliative treatment (n)	1	0	1	n. s.
^{18}F-FET-PET parameters				
SUV_{max}/BG	3.4 ± 0.1	3.0 ± 0.2	3.7 ± 0.1	0.004
SUV_{mean}/BG	2.2 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	0.008
BTV (ml)	20.6 ± 1.9	13.1 ± 2.6	25.5 ± 2.6	0.002
TTP_{min}				
≤12.5 min (n)	72	22	50	0.016
>12.5 min (n)	39	21	18	
not available (n)	10	8	2	

n.s.: *p*-value not significant; radiochemotherapy = radiotherapy with concomitant and adjuvant temozolomide (60 Gy; 75 mg/m^2 body surface, 5/7 days, 6 weeks) according to EORTC/NCIC (*1, 2*); chemotherapy: 19 temozolomide, 4 PCV; radiotherapy dose: 60 Gy.

Table 2 *p*-values for determination of prognostic factors regarding overall survival

Parameter	*p*-value	HR	95% CI
Age (≥ 55 vs. < 55 years)	<0.001	2.38	1.54-3.68
Sex (male vs. female)	0.16	1.35	0.89-2.06
KPS (< 90 vs. ≥ 90)	0.21	1.72	0.74-4.03
WHO (IV° vs. III°)	<0.001	2.56	1.61-4.06
MGMT promoter methylation (no vs. yes)	<0.001	2.89	1.83-4.57
Contrast enhancement (yes vs. no)	0.01	2.32	1.23-4.36
Initial treatment (RT vs. CT vs. RT/CT)	<0.001	1.87	1.38-2.53
Surgical resection (no vs. yes)	0.90	1.03	0.65-1.63
SUV_{max}/BG	0.27	1.12	0.92-1.35
SUV_{mean}/BG	0.42	1.22	0.76-1.96
BTV	0.11	1.01	1.00-1.02
TTP_{min} (≤12.5 min vs. > 12.5 min)	0.001	2.25	1.38-3.67

HR: hazard ratio; CI: confidence interval; RT: radiotherapy; CT: chemotherapy; RT/CT: radiochemotherapy. *p*-values derived from univariate Cox-regression for continuous parameters and Log-rank-test for categoric parameters.

Table 3 *p*-values of prognostic factors in the multivariate analysis regarding overall survival

Parameters	*p*-value	HR	CI
Age (≥ 55 vs. < 55 years)	0.015	1.82	1.12-2.94
MGMT promoter methylation (no vs. yes)	<0.001	2.78	1.71-4.50
TTP_{min} (≤12.5 min vs. > 12.5 min)	0.009	2.04	1.20-3.48
Age (≥ 55 vs. < 55 years)	0.007	1.95	1.02-3.17
MGMT promoter methylation (no vs. yes)	<0.001	2.73	1.70-4.39
WHO (IV° vs. III°)	0.042	1.73	1.02-2.93
Age (≥ 55 vs. < 55 years)	0.026	1.73	1.07-2.68
MGMT promoter methylation (no vs. yes)	<0.001	2.62	1.61-4.27
WHO (IV° vs. III°)	0.150	1.49	0.87-2.56
TTP_{min} (≤12.5 min vs. > 12.5 min)	0.023	1.89	1.09-3.22

HR: Hazard ratio; CI: 95% confidence interval; *p*-values derived from multivariate Cox-regression

The two alternative models, excluding either WHO grade or TTP_{min} as factor, reached a similar fit. In the model with both factors TTP_{min} remains significant.