Abstract. Aim: To evaluate the proliferation activity in gliomas using $^{18}$F-fluorothymidine ($^{18}$F-FLT)-positron emission tomography/computed tomography (PET/CT). Patients and Methods: Samples of 26 tumors were analyzed (mean age=51.6; range=26-72 years; 16 males, 10 females). All examinations were performed using a PET/CT scanner equipped with lutetium oxyorthosilicate (LSO) detectors. All data were acquired with a delay of 15 min, following intravenous application of $^{18}$F-FLT (dosed 2 MBq/kg of body weight). The PET/CT contained CT after intravenous application of iodinated contrast agent and high-resolution brain PET acquired during 15 min in one position. PET/CT was performed before confirmation of the histological diagnosis and the level of $^{18}$F-FLT accumulation was compared to the grading of the tumor evaluated using immunohistochemistry staining of Ki-67. Samples were obtained by stereotactic biopsy (5×) or surgical resection (21×). Results: Five tumors of grade IV, 7 tumors of grade III and 14 tumors of grade II were found. Pre-bioptical discrimination between high-grade and low-grade tumors reached accuracy 92.3% (24/26), sensitivity 92.3% (12/13) and specificity 92.9 (13/14). The mean maximum standardized uptake value (SUV$_{max}$) in high-grade tumors was 2.23, significantly different from low-grade tumors (mean SUV$_{max}$ 0.61, T=7.803, p<0.0001). Conclusion: $^{18}$F-FLT-PET/CT enables to estimate the proliferation activity of glioma before biopsy.

Although the surgical procedures, radiotherapy and chemotherapy keep developing, the prognosis of patients with glial tumors is still largely dependent on their degree of differentiation and remains poor for Grades III and IV. It is rather important to determine the proliferation activity of a tumor in order to be able to estimate the probability of therapeutic success. The immunochemistry staining using the Ki-67 marker became the golden-standard in the assessment of the proliferation activity in human brain tumors. Despite the progress in imaging procedures of magnetic resonance imaging, the ability to detect the acceleration of the tumorous growth at an early stage is still limited from the point of view of its morphological evaluation, diffusion of water molecules and the assessment of blood-brain barrier (BBB) disruption. Even if magnetic resonance spectroscopy is used, the limitations of poorer spatial resolution and relatively uniform spectral findings in all glial tumors have the strongest influence on the discrimination between well- and poorer-differentiated tumors.

Positron emission tomography/computed tomography (PET/CT) with the application of $^{18}$F-fluorothymidine ($^{18}$F-FLT) employs the detection of changes of radio-pharmaceutical accumulation in individual pathological processes for their characterization and differential diagnostics (1, 2). The level of $^{18}$F-FLT accumulation enables to quantify the degree of the proliferation activity of the tissue. A significant improvement of the proliferation activity imaging in tissues has been achieved through the introduction of a new, much more comprehensive imaging method that takes into account the marker of DNA synthesis, $^{18}$F-FLT. $^{18}$F-FLT (3'-deoxy-3'-(18F)-fluorothymidine), a radiolabeled thymidine analog, has been shown to be useful in preoperative imaging of highly proliferating brain, as well as head and neck tumors, lung tumors etc. (3). The $^{18}$F-FLT accumulation in tissues is based on trapped $^{18}$F-FLT inside proliferating cells with active thymidine kinase TK1, a key enzyme in the salvage pathway of DNA synthesis. The intracellular concentration of $^{18}$F-FLT depends on the two-way relatively low selective equilibrative nucleoside plasma membrane human transporters (hNTs) especially hENT1. $^{18}$F-FLT, when impaired, is crossing the BBBand rapidly distributed into the extracellular fluid before taken-up by the nucleoside transporters mentioned above (3).
The present work concerns experience obtained with 18F-FLT – PET/CT in brain pre-biopical discrimination of low- and high-grade gliomas in patients with newly-diagnosed brain tumors.

Patients and Methods

Study population. Our patient sample included 26 patients (mean age=51.6; range=24-72 years; 16 males and 10 females) with cerebral glial tumors with homogenous representation of Grades II–IV diagnosed according to WHO criteria. The evaluation of the sample included images of PET corrected to the attenuation and CT images following the application of iodinated contrast material. During evaluation of PET, the recent magnetic resonance performed within one week on 3T was available, including the images of fractional anisotropy (FA) maps, apparent diffusion coefficient (ADC) maps, magnetic resonance spectroscopy (MRS) and T1-weighted gradient echo images (3D MPRAGE) with an isotropic voxel 1 mm × 1 mm × 1 mm. All patients provided routine informed consent prior to the investigation.

Data acquisition. All imaging investigations were conducted with a Biograph 16 scanner (Siemens, Erlangen, Germany) using a high resolution protocol.

Initially, the CT data were acquired using a protocol with setting of 120 kV, 320 mAs effective, collimation 16×0.75 mm. CT scan was initialized 90 s after intravenous administration of 60 ml of iomeprol (concentration 350 milligram iodine per milliliter, Iomeron 350, Bracco, Milano, Italy). CT data were reconstructed with soft tissue algorithm in width of 3 mm with increment 2 mm useful for attenuation correction of PET, and 2 mm width with 1 mm increment for diagnostic purposes.

Subsequently, the following parameters of PET acquisition were used: field of view in Z axis, 160 mm; time per bed, 15 min; image matrix, 256×256; iterative image reconstruction with 8 iterations and 16 subsets; Gaussian filtration; scatter correction on. The data acquisition started with a 15-min delay after the intravenous 1800-F-FLT application (Radiomedic, Husinec - Rez, Czech Republic) in a dose of 2 MBq/kg of body weight. If no accumulation in brain tissue was found in the initial PET scan, another scan after 30 min was added to assess the late accumulation within the tumorous tissue.

Data analysis. The most frequently performed technique to (semi-) quantitatively evaluate the uptake of FLT in tumors was used, i.e. the calculation of standardized uptake values (maximum FLT-SUV, SUVmax). The circular or elliptic region of interest was placed over the tumorous infiltration in the area with the highest tumor activity, as previously published by Weber et al. (32). Standardized uptake values (SUV) are calculated from each region of interest (ROI) automatically using the formula: SUV=measured activity (Bq/g) × body weight (g)/injected activity (Bq). The tumorous tissue was displayed using fusion of CT and PET, eventually using off-line fusion with previously performed magnetic resonance images.

Histology assessment. Samples of tumorous tissue were taken by stereotactic biopsy (5x) or obtained from surgical resection (21x). As assessed by FLT-PET, immunohistochemical staining of the proliferation- associated antigen Ki-67 was used for comparison to in vivo proliferative activity. At our institution, formalin-fixed, paraffin-embedded sections of resected tumor tissues were stained with the monoclonal Ki-67 specific antibody MIB-1. In each tumor sample, the proliferative activity is calculated as percentage of MIB-1 stained nuclei per total number of nuclei in four representative cross-sections.

Results

Histologically, the tumor samples contained 5 Grade IV tumors (all glioblastomas), 7 Grade III tumors (four anaplastic astrocytomas, one anaplastic oligoastrocytoma, one fibrillary astrocytoma focally upgraded to anaplastic astrocytoma and one gemistocytic astrocytoma focally upgraded to anaplastic astrocytoma) and 14 Grade II tumors (eleven cases of fibrillary astrocytoma, two oligoastrocytoma and one oligodendroglioma) (Figures 1 and 2).

According to our findings on 18F-FLT accumulation, the tumors were divided in three groups: those with SUVmax that reached the level 2.0 and more, those between 1.0 and 2.0 and, finally, those that reached accumulation below the SUVmax of 1.0 SUVmax >2.0 was noted in eleven tumors (ten high-grade tumors and one low-grade tumor), between 1.0 and 2.0 in three tumors (two high-grade tumors and one low-grade tumor) and below 1.0 in twelve tumors (all low-grade tumors). According to the survival pattern obtained (Table I), all patients with tumors with low level of 18F-FLT accumulation, i.e. SUVmax <2.0, survived more than 12 months (including high-grade gliomas), whereas in the group of tumors with accumulation exceeding a SUVmax of 2.0 the 12-month mortality rate was 72.7% (8/11). No additional accumulation of 18F-FLT was noted in the tumorous tissue, when initial PET scan showed uptake of SUVmax below 1.0.

Pre-biopical discrimination between high-grade and low-grade tumors according to the increased accumulation of 18F-FLT when SUVmax ≥2.0 and/or the presence of contrast enhancement on CT reached accuracy 92.3% (24/26), sensitivity 92.3% (12/13) and specificity 92.9 (13/14). The mean SUVmax in high-grade tumors was 2.23 (range, 1.0-2.8) that is significantly different from the levels measured in low-grade tumors (mean SUVmax, 0.61; range, 0.2-2.2). t-test was used T value T=7.803, p<0.0001. (Table I and Figure 3).

Discussion

Shields et al. (1) has reported 18F-FLT as an analog substrate of thymidine, which is intracellularly used during the S-phase of the cell cycle. Due to its rapid uptake in proliferating cells, tumors can be visualized in 15–60 min after intravenous injection of this radiopharmaceutical. Accordingly, the half-life of 18F is 110 min, which is ideal for the PET imaging process. Multiple studies have demonstrated proliferation-dependent uptake of 18F-FLT in tissues with high proliferative activity (4, 5). Intracellularly, 18F-FLT is being present.
predominantly in its phosphorylated form, whereas almost no 
$^{18}$F-FLT is incorporated into the DNA (2). The accumulation 
measurement derived from PET imaging reflects the uptake 
of $^{18}$F-FLT as a function of nucleoside transport and 
consecutive activation of intracellular thymidine by 
monophosphorylation (2). $^{18}$F-FLT is rapidly excreted by the 
kidneys in a non-metabolized form. Relatively high liver 
uptake is regularly detected in humans; liver accumulation is 
related to hepatic glucuronidation of $^{18}$F-FLT (1, 2). In brain, 
the non-glucuronided $^{18}$F-FLT is not able to cross the BBB 
due to the hydrophilic structure of the molecule; however, 
after glucuronidation crossing and delayed accumulation is 
theoretically possible.

### Table I. $^{18}$F-FLT uptake and mortality.

<table>
<thead>
<tr>
<th>SUV$_{\text{max}}$</th>
<th>Grade IV</th>
<th>Grade III</th>
<th>Grade II</th>
<th>All</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>5 (19.2%)</td>
<td>1 (7.1%)</td>
<td>2 (85.8%)</td>
<td>7 (26.9%)</td>
<td>1 (7.1%)</td>
</tr>
<tr>
<td>0-0.99</td>
<td>5 (100%)</td>
<td>2 (71.4%)</td>
<td>12 (46.2%)</td>
<td>26 (100%)</td>
<td>8 (72.7%)</td>
</tr>
<tr>
<td>1.0-1.99</td>
<td></td>
<td>1 (7.1%)</td>
<td>3 (11.5%)</td>
<td>11 (42.3%)</td>
<td></td>
</tr>
<tr>
<td>2.0 and above</td>
<td></td>
<td></td>
<td>1 (7.1%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SUV$_{\text{max}}$, maximum value of standardized uptake value.

Figure 1. Diffuse astrocytoma grade I; A – PET image, B – CT image.

Figure 2. Anaplastic astrocytoma grade III; A – PET image, B – CT image.

Figure 3. Comparison of the $^{18}$F-FLT uptake in low- and high-grade gliomas.
Shinoyamia et al. found that the increased $^{18}$F-FLT accumulation in gliomas reflects changes in ENT (equilibrative nucleoside transporters) expression but they also confirmed that the accumulation of $^{18}$F-FLT depends, even more, on the leakage through the BBB (5). These facts are very well applicable to the use of the $^{18}$F-FLT as a potential biomarker for the discrimination between high- and low-grade gliomas (6, 7). Besides the increased proliferation, the impairment of BBB is the most important property of high-grade gliomas. It seems that the uptake of $^{18}$F-FLT reflects both main behaviors of gliomas: the level of proliferation and BBB disruption, at once. Yang et al. reported that the $^{18}$F-FLT imaging of cell proliferation in lung tumors is also related to the level of angiogenesis assessed by microvessel density using the CD34 score. $^{18}$F-FLT leakage through the BBB can be explained by the same mechanism, as glioblastomas and anaplastic astrocytomas are typical tumors with extremely pronounced neoangiogenesis (6, 8-10). Our own results support the aforementioned mechanism because no tumor showed a delayed uptake of $^{18}$F. Hepatic glucuronidation facilitates the $^{18}$F-FLT molecule to cross the BBB but the uptake is then depending on the population of cells in S-phase.

Obviously, the preferred technique to assess the uptake of $^{18}$F-FLT in brain tumors is the calculation of standardized uptake values (mean or maximum FLT-SUV). There are more complex approaches available, such as kinetic modeling, which are able to generate further precise information on tracer kinetics, such as flux or transfer constant and can correct the background activity within blood vessels. These methods, however, although more accurate, do not provide clinically important information related to the therapy approaches. Since the superiority of these techniques over a simple calculation of SUV values has not been fully demonstrated so far, the above listed protocol for tracer quantification used in our institution is still widely recommended. Our own results support the role of this approach for a simple, semi-quantitative analysis of $^{18}$F-FLT uptake for the estimation of prognosis and potential effect of surgical resection. Although the documented differences between $^{18}$F-FLT uptakes in high- and low-grade gliomas were previously published. The possibility to discriminate those tumors, even to estimate the survival according to the level of accumulation of $^{18}$F-FLT. Despite the histological diagnosis, a SUV$_{max}$ level below 2.0 could be used as an important predictor of survival. SUV$_{max}$ as a predictor of mortality could be documented in the case of patient with histologically-proven diagnosis based on the stereotactic biopsy – even though the histological diagnosis was set as fibrillary astrocytoma, the patient died due to the rapid tumor progression within three months.

Some tumors present heterogeneity in $^{18}$F-FLT accumulation indicating the presence of different cellular populations with different level of BBB disruption and cellular proliferation and, thus, the different growth potential within one tumor (1). The locally elevated $^{18}$F-FLT accumulation indicated the optimal site to obtain a sample using stereotactic biopsy. PET images could be used in the planning of targeted-biopsy (10). This approach was probably responsible for the identification of most tumors in the blastematos turn over.

Conclusion

PET/CT with the application of $^{18}$F-FLT could play an important role in pre-biopical assessment of the glioma behavior. The high level of $^{18}$F-FLT uptake reflects the higher level of neoangiogenesis in the tumor, as well as the increase in proliferation activity. Our in vivo observations of tumorous heterogeneity and its confirmation by histological assessment are supporting the theories of intra-tumorous evolution as a motion force in glioma development.

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References


