Imaging Tryptophan Metabolism in Human Brain Tumors

Edit Bosnyák, MD

Ph.D. Thesis

Mentor: Prof. Csaba Juhász, MD, PhD

Co-Mentor: Zoltán Pfund, MD, PhD

Doctoral School Director: Prof. Sámuel Komoly, MD, DSc

University of Pécs, Faculty of Medicine, Department of Neurology, Hungary

Clinical Neuroscience Doctoral School

Wayne State University School of Medicine, Detroit, Michigan;

PET Center and Translational Imaging Laboratory, Children's Hospital of Michigan, Detroit, USA

Pécs, 2018

LIST OF CONTENTS

ABBREVIATIONS

AMT: ^{11}C-alpha-methyl-L-tryptophan

BDI-II: Beck Depression Inventory-II

CNS: central nervous system

EGFR: epidermal growth factor receptor

FLAIR: fluid-attenuated inversion recovery

GBM: glioblastoma

IDH1: isocitrate dehydrogenase1

IDO1/2: indoleamine 2,3-dioxygenase 1/2

KMO: kynurenine 3-monooxygenase

KP: kynurenine pathway

KYNU: kynureninase

MGMT: O^6-methylguanine-DNA methyltransferase

MRI: magnetic resonance imaging

PET: positron emission tomography

RANO: Response Assessment in Neuro-Oncology

TDO2: tryptophan 2,3-dioxygenase 2

WHO: World Health Organization

I. INTRODUCTION

Brain tumors are relatively rare in adults but represent the most common solid tumors in children. In all ages, brain tumors carry a significant mortality, and, therefore, are considered to be a major health care issue. The majority of newly-diagnosed brain masses are metastatic tumors, while the rest represent a variety of primary central nervous system (CNS) tumors. There are a few known risk factors associated with brain tumors, such as ionizing radiation and genetic predisposition. Genetic susceptibility for brain tumors may exist, however, the majority of brain tumors are sporadic. On the other hand, autoimmune conditions and allergies are inversely correlated with glioma risk. The most common primary brain tumor is meningioma, followed by glioblastoma. Based on Surveillance Epidemiology and End Results Program, 2016, in the adult population above age 40 years, the average annual age-adjusted incidence rate of primary CNS tumors is 40.10/100,000. The 5-year relative survival of primary malignant CNS tumors between 1995-2013 was 34.7% (higher in females), but it was modified significantly by age, histology and clinical behavior, while in non-malignant CNS tumors, 5-year survival was 90.4% in US.

In general, the most common clinical manifestation of brain tumors include seizure, focal neurological deficit, altered behavior, cognitive impairment, but mood disturbance and depression are also common co-morbidities among these patients. In gliomas, the age of the patients and also tumor grade have a significant effect for the presenting symptoms. Seizure can be a common symptom in patients with low-grade glioma, while focal neurological and cognitive deficits are more common in high-grade glioma patients. However, in some cases, the initial manifestation of brain tumors is different psychiatric symptoms, such as depression, apathy, personality changes, anxiety, etc. Meningiomas are mainly solitary tumors, and approximately 2-3% of the population has an incidental asymptomatic meningioma. One of the most common manifestation of meningiomas is seizure, which occurs in 13-60% of affected patients.

Until the recent revision in 2016, the classification of CNS tumors mainly relied on conventional histopathologic characteristics. However, in the recently released 2016 World Health Organization (WHO) classification of CNS tumors, for the first time, molecular markers have been incorporated in addition to conventional histology to classify primary brain tumors. This formed a new concept as to how CNS tumor diagnoses should be established using recently recognized molecular characteristics. This classification also added some newly recognized neoplasms and deleted some others that have no longer diagnostic and/or biological relevance (e.g., the use of oligoastrocytoma as a separate entity is now discouraged, because astrocytomas and oligodendrogliomas can be distinguished by specific molecular markers). Other changes included the addition of brain invasion as a criterion for atypical meningioma and the introduction of a soft tissue-type grading system.

I.1. Neuroimaging in brain tumors

Magnetic resonance imaging (MRI) with contrast administration is the standard clinical method for initial diagnosis of brain tumors. MRI plays an important role in differentiation, presurgical evaluation, treatment planning, and post-treatment follow-up. However, conventional MRI has a limited ability to differentiate low-grade from high-grade (infiltrative) tumors and also recurrent/progressing brain tumors from radiation injury. Advanced MRI techniques, such as diffusion-weighted imaging (DWI), perfusion-weighted imaging (PWI) and MR spectroscopy (MRS) add important information for tumor diagnosis and management and are also promising techniques in the identification of potential molecular characteristics of brain tumors. Molecular imaging with positron emission tomography (PET) also plays an increasing role in selected subgroups of patients with pre- and post-treatment brain tumors, both in adults and in pediatric brain tumors.

I.1.1. Conventional MRI in brain tumors

Low-grade gliomas are typically seen as a non-enhancing lesion on MRI, while anaplastic glioma (Grade III) and glioblastoma (GBM) (Grade IV) often present as contrast-enhancing lesions on MRI. The typical radiographic features of GBM on post-contrast T1 images include thick irregular ring of heterogeneous enhancement surrounding a central necrosis, often with a larger area of peritumoral hyperintensity; the latter more evident on fluid-attenuated inversion recovery (FLAIR) and T2-weighted sequences, representing the region of tumor infiltration and vasogenic edema. Meningiomas can be diagnosed on pre- and post-contrast T1-weighted, T2-weighted and FLAIR images; however, MRI has a limited ability to differentiate low-grade from high-grade meningiomas or tumor tissue from non-specific tissue changes. In current clinical practice, conventional MRI, such as T2-weighted, FLAIR, and, particularly, pre- and post-contrast T1-weighted MRI are the gold standard diagnostic tools not only in the initial diagnosis but also in post-treatment evaluation of brain tumors, where pseudo-progression and pseudo-response (for example, after treatment with antiangiogenic agents) are a common challenge. Progressive disease may be diagnosed by Response Assessment in Neuro-Oncology (RANO) criteria in high-grade gliomas (see more details in the thesis).

I.1.2. Advanced MRI in brain tumors

In the last decade, advanced MRI techniques have been under intense investigations as promising diagnostic tools in both newly diagnosed and previously treated brain tumors. DWI, PWI and MRS can provide detailed physiologic information about several tumor characteristics, such as vascularisation, microperfusion, and cellularity, and they have shown a promise in the post-treatment evaluation of malignant gliomas. DWI can estimate tumor density and can be useful to differentiate non-enhancing tumor area from peritumoral edema in white matter. PWI can predict

the tumor grade based on the assessment of angiogenesis and blood brain barrier permeability; also it might be an important diagnostic tool during antiangiogenic therapy. In addition, PWI can play a significant role in neuro-oncology as a noninvasive diagnostic tool for prognosis and response to therapy. MR spectroscopy measures tumor-related changes of various metabolites, such as choline, lactate, creatine, N-acetylaspartate (NAA), which can be associated with the tumor grade. Changes in these metabolites can also estimate the proliferation rate of tumor cells (based on choline/NAA) and presence of necrosis (lipids or lactate peak).

I.1.3. PET imaging in neuro-oncology

In neuro-oncology, molecular imaging with PET can play an important additional role in diagnosis and management. PET can detect and characterize different types of tumors based on their metabolic properties, such as altered glucose, nucleoside and amino acid metabolism.

I.1.3.1. FDG-PET: 2-deoxy-2[^{18}F]fluoro-D-glucose (FDG) PET has evolved over the past three decades into a key clinical PET modality in detecting both intra- and extracranial tumors. In neuro-oncology, the primary role of FDG-PET is differentiation of malignant from benign lesions and distinguishing recurrent tumors from radiation injury. The main advantages of FDG include its relatively long half-life (110 minutes) and its automated radio-synthesis.

I.1.3.2. Amino-acid PET: Most tumors have higher amino acid uptake and metabolism than normal cells. This difference is the basis of the high sensitivity of amino acid PET in cancer imaging. The most widely tested amino acid PET tracers in brain tumor imaging include L-[methyl-^{11}C]methionine (MET), ^{18}F-fluoroethyl-tyrosine (FET), and ^{18}F-fluoro-L-dihydroxy-phenylalanine (FDOPA); the group at Wayne State University has also introduced the human use of ^{11}C-alpha-methyl-L-tryptophan (AMT) in cancer imaging. MET is the most studied tracer, although it is labeled with the short half-life (20 min) carbon-11 positron-emitting isotope. FET is labeled with ^{18}F, which has a 110 min half-life that is more suitable for routine clinical applications. Both MET and FET-PET can detect and differentiate newly diagnosed and recurrent gliomas, and provide useful information in initial treatment planning and response monitoring. FDOPA, originally developed to measure dopamine synthesis, is used in a few centers for brain tumor imaging; it has amino acid kinetic characteristics similar to FET.

I.1.3.3. AMT-PET: AMT was developed originally to estimate brain serotonin synthesis rates. Increased cortical AMT uptake can also to identify epileptogenic foci in patients with drug resistant epilepsy. In initial studies of AMT-PET in brain tumors, high tryptophan uptake was detected in a variety of low-grade and high-grade brain tumors. In addition to measuring static tumoral AMT uptake, tracer kinetic analysis was found to be useful in differentiation low-grade tumor types, estimate tumor proliferative activity, and differentiate recurrent tumor from radiation necrosis. We have also demonstrated that combination of AMT-PET with advanced MRI, such as diffusion tensor imaging, can facilitate non-invasive estimation of tumor

cellularity. Tryptophan and AMT is mostly transported into brain tumor tissue via L-type amino acid transporter. However, AMT, unlike tryptophan, is not incorporated into proteins, because of the added methyl group in the alpha position. Tryptophan can be metabolized via the immunomodulatory kynurenine pathway (KP), which plays a key role in tumoral immune tolerance.

II. OBJECTIVES

The overall aim of our studies was to explore the potential clinical use of AMT-PET in both newly diagnosed and recurrent brain tumors. We focused on applications where amino acid PET has not been used or validated before. The objectives of four published studies are summarized below.

In Study 1, we had two main goals: i. To evaluate if prognostic molecular markers in primary (IDH1 wild-type) glioblastomas (GBMs) are associated with a specific pattern of amino acid uptake or metabolism on PET imaging and/or magnetic resonance imaging (MRI) variables; ii. To determine if pre-treatment AMT uptake measured by PET has a prognostic value for overall survival in the same group.

In Study 2, we evaluated the clinical value of high AMT uptake in brain regions outside the contrast-enhancing tissue in post-treatment glioblastomas. Specifically, we tested if non-enhancing brain regions showing increased AMT uptake predict the spatio-temporal pattern of subsequent tumor progression during imaging follow-up.

In Study 3, we explored the potential role of abnormal tryptophan metabolism in brain tumor-associated depression. The overall goal was to determine if abnormal brain tryptophan metabolism measured in non-tumoral brain regions, measured by PET, could be an imaging biomarker for brain tumor-associated depression.

In Study 4, we evaluated mechanisms and potential clinical significance of abnormal tryptophan uptake and metabolism in WHO grade I–III meningiomas using AMT-PET with detailed tracer kinetic analysis.

III. SUMMARY OF THE FOUR STUDIES

Our multimodal imaging studies included groups of adult patients with a variety of pre- and post-treatment brain tumors. All studies were approved by the Institutional Review Board of Wayne State University, and written informed consent was obtained from all participants. In all studies, the statistical analysis was performed using IBM SPSS Statistics for Windows (IBM Corp., Armonk, NY), Version 19.0, 21.0., or 23.0. A p value of <0.05 was considered significant.

III.1. ***Study 1 - Prognostic Molecular and Imaging Biomarkers in Primary Glioblastoma (Bosnyák et al. Clin Nucl Med, 2017)***

III.1.1. *Purpose of the study:* Several molecular glioma markers (including isocitrate dehydrogenase 1 [IDH1] mutation, amplification of the epidermal growth factor receptor [EGFR], and methylation of the O^6-methylguanine-DNA methyltransferase [MGMT] promoter) have been associated with glioblastoma survival. In this study, we examined the association between tumoral amino acid uptake, molecular markers, and overall survival in patients with IDH1 wild-type (primary) glioblastoma.

III.1.2. *Subjects and Methods:* Twenty-one patients (14 males, mean age: 62 years) with newly diagnosed IDH1 wild-type glioblastomas underwent presurgical MRI and AMT-PET scanning. At initial surgery, 13 patients had gross total tumor resection, while 8 patients had subtotal resection. The median survival time was 14.8 months, and 13 of the 21 patients (62%) had >1-year survival.

MRI characteristics (T2- and T1-contrast volume), tumoral tryptophan uptake, PET-based metabolic tumor volume, and PET kinetic variables were correlated with prognostic molecular markers (EGFR and MGMT) and overall survival.

III.1.3. *Results:* EGFR amplification was associated with lower T1-contrast volume (p=0.04) as well as lower T1-contrast/T2 volume (p=0.04) and T1-contrast/PET volume ratios (p=0.02). Tumors with MGMT promoter methylation showed lower metabolic volume (p=0.045) and lower tumor/cortex AMT unidirectional uptake ratios than those with unmethylated MGMT promoter (p=0.009). While neither EGFR amplification nor MGMT promoter methylation was significantly associated with survival, high AMT tumor/cortex uptake ratios on PET were strongly prognostic for longer survival (hazards ratio,30; p= 0.002). Estimated mean overall survival was 26 months in patients with high versus 8 months in those with low tumor/cortex AMT uptake ratios.

III.1.4. *Conclusions:* The results demonstrate specific MRI and amino acid PET imaging characteristics associated with EGFR amplification and MGMT promoter methylation in patients with primary glioblastoma. High tryptophan uptake on PET may identify a subgroup with prolonged survival.

III.2. *Study 2 - Tryptophan PET predicts spatial and temporal patterns of post-treatment glioblastoma progression detected by contrast-enhanced MRI (Bosnyák et al., J Neurooncol, 2016)*

III.2.1. *Purpose of the study:* Amino acid PET is increasingly utilized for the detection of recurrent gliomas. Increased amino acid uptake is often observed outside the contrast-enhancing brain tumor mass. In this study, we evaluated if MRI non-enhancing PET+ regions could predict spatial and temporal patterns of subsequent MRI progression in previously treated glioblastomas.

III.2.2. *Subjects and Methods:* Twelve patients (6 males, mean age: 61 years) with a contrast-enhancing area suspicious for glioblastoma recurrence on MRI underwent PET scanning with AMT-PET. Brain regions showing increased AMT uptake in and outside the contrast-enhancing volume were objectively delineated to include high uptake consistent with glioma (using an uptake threshold defined by previous studies). Volume and tracer uptake of such non-enhancing PET+ regions were compared to spatial patterns and timing of subsequent progression of the contrast-enhancing lesion, as defined by serial surveillance MRI.

III.2.3. *Results:* Non-enhancing PET+ volumes varied widely across patients and extended up to 24 mm from the edge of MRI contrast enhancement. In 10 patients with clear progression of the contrast-enhancing lesion (1-17 months after the AMT-PET scan), the non-enhancing PET+ volumes predicted the location of new enhancement, which extended beyond the PET+ brain tissue in six and in four patients the Gad+ volume mirrored the PET+ volume with no or minimal extension beyond the PET+ area. In two patients, with no PET+ area beyond the initial contrast enhancement, MRI remained stable. There was a negative correlation between AMT uptake in non-enhancing brain and time to subsequent progression ($r=-0.77$, $p=0.003$).

III.2.4. *Conclusions:* Amino acid PET imaging could complement MRI not only for detecting glioma recurrence but also predicting the location and timing of subsequent tumor progression. This could support decisions for surgical intervention or other targeted therapies for recurrent gliomas.

III.3. *Study 3 - Imaging cerebral tryptophan metabolism in brain tumor-associated depression (Bosnyák et al., EJNMMI Research, 2015)*

III.3.1. *Purpose of the study:* Depression affects patients with brain tumors of all grades and types and is associated with impaired quality of life and shorter survival. Altered metabolism of tryptophan to serotonin and kynurenine metabolites may play a role in tumor-associated depression. Our recent studies with AMT-PET in brain tumor patients indicated abnormal tryptophan metabolism not only in the tumor mass but also in normal-appearing contralateral brain. In this study, we explored if tryptophan metabolism in such brain regions is associated with depression.

III.3.2. *Subjects and Methods:* The study included 21 patients (13 males, mean age: 57 years) with a variety brain tumor (10 meningiomas, 8 gliomas, and 3 brain metastases). Four patients had resective surgery before with or without chemoradiation. None of the 21 patients had a history of clinical depression and were not on any antidepressant medication at the time of AMT-PET. Karnosfky performance status score in all patients was 70 or higher. MRI and AMT-PET images were co-registered, and AMT kinetic parameters, including volume of distribution (VD', an estimate of net tryptophan transport) and K (unidirectional uptake, related to tryptophan metabolism), were measured in the tumor mass and in unaffected cortical and subcortical regions contralateral to the tumor. All patients were screened for depression on the day of the PET scan using the Beck Depression Inventory-II (BDI-II), and the BDI-II scores were correlated with tumor size, grade, type, and AMT-PET variables.

III.3.3. *Results:* The mean BDI-II score was 12±10 (range: 2–33); clinical levels of depression were identified in seven patients (33 %). High BDI-II scores were most strongly associated with high thalamic AMT K values both in the whole group (Spearman's rho=0.63, p=0.004) and in the subgroup of 18 primary brain tumors (r=0.68, p=0.004). Frontal and striatal VD' values were higher in the depressed subgroup than in non-depressed patients ($p<0.05$); the group difference was even more robust when moderately/severely depressed patients were compared to patients with no/mild depression (frontal: p=0.005; striatal: $p< 0.001$). Tumor size, grade, and tumor type were not related to depression scores.

III.3.4. *Conclusions:* Abnormalities of tryptophan transport and metabolism in the thalamus, striatum, and frontal cortex, measured by PET, are associated with depression in patients with brain tumor. These changes may indicate an imbalance between the serotonin and kynurenine pathways and serve as a molecular imaging marker of brain tumor-associated depression.

III.4. ***Study 4 - Molecular imaging correlates of tryptophan metabolism via the kynurenine pathway in human meningiomas (Bosnyák et al., Neuro-Oncology, 2015)***

III.4.1. *Purpose of the study:* Increased tryptophan metabolism via the kynurenine pathway (KP) is a key mechanism of tumoral immune suppression in gliomas. However, details of tryptophan metabolism in meningiomas have not been elucidated. In this study, we evaluated in vivo tryptophan metabolism in meningiomas and compared it with gliomas using AMT-PET. We also explored expression patterns of KP enzymes in resected meningiomas.

III.4.2. *Subjects and Methods:* Forty-seven patients (26 males, age range: 10-91 years) with MRI-detected meningioma (n=16) and glioma (n=21 grade II and n=10 grade III glioma) underwent presurgical AMT-PET scanning. Tumoral AMT uptake and tracer kinetic parameters (including K and k_3'evaluating unidirectional uptake and trapping, respectively) were measured, correlated with meningioma grade, and compared between meningiomas and gliomas. Patterns

of KP enzyme (3 initial enzymes [IDO1, IDO2 and TDO2] and 2 downstream enzymes [KMO, KYNU]) expression were assessed by immunohistochemistry in all meningiomas.

III.4.3. *Results:* Meningioma grade showed a positive correlation with AMT k_3' tumor/cortex ratio (r=0.75, p=0.003), and this PET parameter distinguished grade I from grade II/III meningiomas with 92% accuracy. Kinetic AMT parameters could differentiate meningiomas from both low-grade gliomas (97% accuracy by k_3'ratios) and high-grade gliomas (83% accuracy by K ratios). Among 3 initial KP enzymes, TDO2 showed the strongest immunostaining, particularly in grade I meningiomas. TDO2 also showed a strong negative correlation with AMT k_3' ratios (p=0.001).

III.4.4. *Conclusions:* PET imaging of tryptophan metabolism can provide quantitative imaging markers for differentiating grade I from grade II/III meningiomas. TDO2 may be an important driver of *in vivo* tryptophan metabolism in these tumors. These results can have implications for pharmacological targeting of the KP in meningiomas.

IV. SUMMARY

New Findings:

- Link between prognostic genetic glioma biomarkers (such as IDH, MGMT, EGFR) and tumoral amino acid uptake in glioblastomas. Specific MRI and AMT-PET characteristics associated with prognostic molecular markers in IDH1 wild-type glioblastoma.
- High AMT tumor/cortex uptake ratio was a strong prognostic imaging marker associated with a markedly longer survival in IDH1 wild-type glioblastomas.
- AMT-PET could complement conventional contrast-enhanced MRI not only for detecting glioma recurrence but also predicting the location and timing of subsequent tumor progression.
- Negative correlation between the AMT SUV and time to T1-Gad MRI progression, indicating that higher AMT uptake in non-enhancing areas could predict earlier glioma progression.
- Link between imaging of cerebral tryptophan metabolism in brain tumor-associated depression. Abnormalities of tryptophan transport and metabolism in the thalamus, striatum and frontal cortex, measured by PET, are associated with depression in patients with brain tumor.
- High accuracy of AMT-PET to distinguish grade I versus grade II-III meningiomas.
- AMT-PET kinetic parameters also showed a striking difference between meningiomas and both grade II and grade III gliomas. Meningiomas could be differentiated from high-grade gliomas with 83% accuracy and with 97% accuracy from low-grade gliomas.

My studies have built upon previous observations demonstrating that PET imaging with the tryptophan derivative AMT is useful in evaluation of various brain tumors. The four studies summarized above included a diverse group of brain tumors and described several novel, previously unexplored clinical applications of this PET modality in both pre- and post-treatment assessments. These studies illustrate the versatility of tryptophan PET imaging and also highlight the additional information we can derive from tracer kinetic analysis and multi-modality imaging by combining quantitative PET and MRI variables. The results provided novel data for the potential utility of this imaging approach to assess molecular characteristics of various brain tumors, obtain objective prognostic biomarkers, and understand potential mechanisms of tumor-associated depression. While these data are very promising, it should be noted that AMT-PET is not widely used in clinical radiology mostly because of the short half-life of C-11 (20 min). In order to overcome this limitation, there have been recent efforts to develop novel, F-18 labeled tryptophan analogs, to image tryptophan transport and metabolism via the kynurenine pathway. The initial results are promising, e.g., with the synthesis and use of 1-(2-^{18}-fluoroethyl)-L-tryptophan, which showed robust tumor uptake, and kinetics similar to AMT in patient-derived xenograft models. While testing of other F-18-labeled radiotracers are also under investigation, the favorable clinical results with AMT-PET, outlined in this thesis, will provide the motivation for further work in this field toward improved, clinically feasible molecular imaging methods for the evaluation of metabolism of tryptophan (and other amino acids) in human cancers. Further, multimodal studies incorporating advanced quantitative MRI with PET imaging are also expected to improve pre- and post-treatment assessment of brain tumors in the near future.

V. PUBLICATIONS

V.1. Peer-reviewed publications related to this thesis

1. **Bosnyák** E, Kamson DO, Guastella AR, Varadarajan K, Robinette NL, Kupsky WJ, Muzik O, Michelhaugh SK, Mittal S, Juhász C. Molecular imaging correlates of tryptophan metabolism via the kynurenine pathway in human meningiomas. Neuro Oncol. 2015; 17:1284-92. (**IF 7.37**)

2. **Bosnyák** E, Kamson DO, Behen ME, Barger GR, Mittal S, Juhász C. Imaging cerebral tryptophan metabolism in brain tumor-associated depression. EJNMMI Res. 2015; 5:56. (**IF: 1.761**)

3. **Bosnyák** E, Kamson DO, Robinette NL, Barger GR, Mittal S, Juhász C. Tryptophan PET predicts spatial and temporal patterns of post-treatment glioblastoma progression detected by contrast-enhanced MRI. J Neurooncol. 2016; 126:317-25. (**IF: 2.754**)

4. **Bosnyák** E, Michelhaugh SK, Klinger NV, Kamson DO, Barger GR, Mittal S, Juhász C. Prognostic Molecular and Imaging Biomarkers in Primary Glioblastoma. Clin Nucl Med, 2017; 42:341-7. (**IF: 4.563**)

5. Juhász C, **Bosnyák** E. PET and SPECT studies in children with hemispheric low-grade gliomas. Childs Nerv Syst. 2016; 32:1823-32. (**IF: 1.08**)

6. Erdélyi-Bótor S, Komáromy H, Kamson DO, Kovács N, Perlaki G, Orsi G, Molnár T, Illes Z, Nagy L, Kéki S, Deli G, **Bosnyák** E, Trauninger A, Pfund Z. Serum L-arginine and dimethylarginine levels in migraine patients with brain white matter lesions. Cephalalgia. 2017; 37:571-580. (**IF: 6.05**)

7. Jeong JW, Juhász C, Mittal S, **Bosnyák** E, Kamson DO, Barger GR, Robinette NL, Kupsky WJ, Chugani DC. Multi-modal imaging of tumor cellularity and tryptophan metabolism in human gliomas. Cancer Imaging. 2015; 15:10. (**IF: 1.47**)

8. **Bosnyák** E, Barger GR, Michelhaugh S, Robinette NL, Amit-Yousif A, Mittal S, Juhász C. Amino Acid PET Imaging of the Early Metabolic Response during Tumor-Treating Fields (TTFields) Therapy in Recurrent Glioblastoma. Clin Nucl Med. 2018; 43:176-179. (**IF: 4.563**)

V.2. Peer-reviewed publications not related to this thesis

1. **Bosnyák** E, Behen ME, Guy WC, Asano E, Chugani HT, Juhász C. Predictors of Cognitive Functions in Children With Sturge-Weber Syndrome: A Longitudinal Study. Pediatr Neurol. 2016; 61:38-45. (**IF: 1.866**)

2. **Bosnyák E**, Herceg M, Pál E, Aschermann Z, Janszky J, Késmárki I, Komoly S, Karádi K, Dóczi T, Nagy F, Kovács N. Are branded and generic extended-release ropinirole formulations equally efficacious? A rater-blinded, switch-over, multicenter study. Parkinsons Dis. 2014; 2014:158353. (**IF: 2.01**)

3. Deli G, **Bosnyak E**, Pusch G, Komoly S, Feher G. Diabetic Neuropathies: Diagnosis and Management. Neuroendocrinology. 2014, Vol.98,No.4. (**IF: 4.373**)

4. Karádi K, Lucza T, Aschermann Z, Komoly S, Deli G, **Bosnyák E**, Acs P, Horváth R, Janszky J, Kovács N. Visuospatial impairment in Parkinson's disease: the role of laterality. Laterality. 2015; 20:112-27. **(IF: 1.312**)

5. Szapary L, Fehér G, **Bosnyák E**, Deli G, Csécsei P. Effective, safe stroke prevention with novel oral anticoagulants in patients with atrial fibrillation. Focus on dabigatran. Ideggy Sz. 2013; 66: 165-74. (**IF: 0.343**)

6. Deli G, Balás I, Komoly S, Dóczi T, Janszky J, Illés Z, Aschermann Z, Tasnádi E, Nagy F, Pfund Z, Bóné B, **Bosnyák E**, Kuliffay Z, Szijjartó G, Kovács N. Treatment of dystonia by deep brain stimulation: a summary of 40 cases. Ideggy Sz. 2012; 65:249-60. (**IF: 0.348**)

7. Toth P, KoIler A, Pusch G, **Bosnyak E**, Szapary L, Komoly S, et al. Microalbuminuria, indicated by total versus immunoreactive urinary albumins, in acute ischemic stroke patients. J Stroke Cerebrovasc Dis. 2011; 20:510-6. (**IF: 1.68**)

8. Deli G, Balás I, Komoly S, Dóczi T, Janszky József, Aschermann Z, Nagy F, **Bosnyák E**, Kovács N. Earlier and more efficiently: The role of Deep Brain Stimulation in Parkinson's Disease: preserving the working capability. Ideggyogy Sz. 2015; 68:384-90. (**IF: 0.376**)

9. Horváth K, Aschermann Z, Ács P, **Bosnyák E**, Deli G, et al. Validation of the Hungarian Unified Dyskinesia Rating Scale. Ideggyogy Sz. 2015; 68:183-8. (**IF: 0.376**)

10. Deli G, Aschermann Z, Ács P, **Bosnyák E**, Janszky J, et al. Bilateral Subthalamic Stimulation can Improve Sleep Quality in Parkinson's Disease. J Parkinsons Dis. 2015; 5:361-8. (**IF: 3.015**)

11. Kovács N, Aschermann Z, Ács P, **Bosnyák E**, Deli G, Janszky J, Komoly S. The impact of levodopa-carbidopa intestinal gel on health-related quality of life in Parkinson's disease. Ideggyogy Sz. 2014; 67:245-50. (**IF: 0.386**)

12. Horváth K, Aschermann Z, Acs P, **Bosnyák E**, Deli G, et al. Is the MDS-UPDRS a Good Screening Tool for Detecting Sleep Problems and Daytime Sleepiness in Parkinson's Disease? Parkinsons Dis. 2014; 2014:806169. (**IF: 2.01**)

V.3. Presentations related to this thesis

Bosnyák E, Kamson D, Muzik O, Mittal S, Juhász C. Characterization of meningiomas by kinetic analysis of alpha[C-11]-methyl-L-tryptophan PET. Presented at the 21st Annual Meeting of the Organization for Human Brain Mapping, Honolulu, HI, June 14-18, 2015.

Bosnyák E, Kamson D, Behen M, Barger G, Mittal S, Juhász C. Correlation of cerebral tryptophan metabolism with brain tumor-associated depression: A PET study. Presented at the 21st Annual Meeting of the Organization for Human Brain Mapping, Honolulu, HI, June 14-18, 2015.

Bosnyák E, Kamson D, Robinette N, Barger G, Mittal S, Juhász C. Multimodal imaging of spatial patterns of post-treatment glioblastoma progression. Neuro-Oncology 2015; 17(suppl 5):v162. doi:10.1093/neuonc/nov225.38. Presented at the 20th Annual Scientific Meeting of the Society for Neuro-Oncology, San Antonio, TX, November 19-22, 2015.

Juhász C, **Bosnyák E**, Kamson D, Barger G, Behen M, Mittal S. Imaging cerebral tryptophan metabolism in brain tumor-associated depression. Neuro-Oncology 2015; 17 (suppl 5): v162. doi: 10.1093/neuonc/nov225.37. Presented at the 20th Annual Scientific Meeting of the Society for Neuro-Oncology, San Antonio, TX, November 19-22, 2015.

Bosnyák E, Michelhaugh SK, Klinger NV, Barger GR, Mittal S, Juhász C. Amino acid metabolism measured by PET is associated with specific molecular biomarkers in primary gliobastoma. Neuro-Oncology 2016; 18(suppl 6): vi132. Presented at the 21st Annual Scientific Meeting of the Society for Neuro-Oncology, Scottsdale, AZ, November 17-21, 2016.

Juhász C, Mittal S, Shah VB, **Bosnyák E**, Barger GR. Imaging the early metabolic response during tumor-treating fields therapy in recurrent glioblastoma. Neuro-Oncology 2016; 18(Suppl 6): vi132. Presented at the 21st Annual Scientific Meeting of the Society for Neuro-Oncology, Scottsdale, AZ, November 17-21, 2016.

Bosnyák E, John F, Robinette NL, Yousif A, Barger GR, Mittal S, Juhász C. Amino acid PET and perfusion MRI in contrast-enhancing and non-enhancing regions of glioblastomas. Neuro-Oncology, 2017; 19(suppl 6): vi161. Presented at the 22nd Annual Scientific Meeting of the Society for Neuro-Oncology, San Francisco, CA, November 16-19, 2017.

VI. ACKNOWLEDGEMENT

My studies were supported by research grants from the National Cancer Institute (R01 CA123451) and from the National Institute of Neurological Disorders and Stroke (R01 NS04192222). Tissue sections were prepared by the Biobanking and Correlative Sciences Core, which is supported in part by National Institutes of Health Center Grant P30 CA022453 to the Karmanos Cancer Institute at Wayne State University (WSU).

My work could not have been carried out without the help of many people, to whom I am really grateful:

First of all, I would like to thank to my supervisor, Professor Csaba Juhász for supporting me, teaching me the basics of neuroimaging in neuro-oncology, especially AMT-PET, and for his continuous support during my years at WSU, and also for his advise in summarizing my thesis.

I also thank to my co-mentor, Dr. Zoltán Pfund for supporting my trip to the US, encouraging me during those years, and advising me with my work on my thesis.

I am grateful to Professor Sámuel Komoly and Professor József Janszky, who also supported me when I got this opportunity.

I would like to say a big thank you to all those colleagues and staff who supported my work at Wayne State University: Prof. Sandeep Mittal, MD (Chair, Neurosurgery), and Geoffrey Barger, MD (Neurology), Co-directors of the WSU/Karmanos Cancer Institute Neuro-Oncology Multidisciplinary Team; Natasha L. Robinette, MD, and Alit Yousif, MD (Radiology), for reviewing the clinical MRI scans; Thomas Mangner, PhD (PET Center, Children's Hospital of Michigan) for performing AMT radiochemistry; Cathie Germain, MA, Cynthia Burnett, BA, and Kelly Forcucci, RN, for assisting patient recruitment and scheduling; and Ms. Lynda Ferguson, for her continuous support in human subjects research administration.

I also thank James Janisse, PhD, for assisting with the statistical analysis, and Sharon Michelhaugh, PhD, who supervised tumor tissue studies at the WSU Neuro-Oncology Research Laboratory.

I am very grateful to the entire staff at the PET Center, Children's Hospital of Michigan, who provided invaluable technical help in performing the PET scans and for constantly advising and helping me.

Special thanks to all of my friends in the US and Hungary for their continuous support.

Finally, I would like to express my gratitude to my parents, my sisters, and my grandmother for their love and for supporting and encouraging me through all these years. Leaving them behind and living in the US was a big decision, but one of the best ones in my life. My family always supported and stood by me in this endaveour, and I cannot be grateful enough to them.