MULTIMODALITY NEUROIMAGING IN MIGRAINE AND BRAIN TUMORS

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Ph.D. Thesis

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This thesis provides a detailed overview of the original research work I’ve done investigating headache magnetic resonance imaging (MRI) at the Department of Neurology of University of Pécs, and multimodality brain tumor imaging at the Translational Imaging and PET Center at Wayne State University, USA. This endeavor could not have been fruitful without the tremendous help, guidance and encouragement I’ve received from my mentors Drs. Pfund and Juhász and from my colleagues and wife, for which I am grateful.
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ABBREVIATIONS
(In alphabetical order)

AMT: alpha-[\(^{11}\)C]-methyl-L-tryptophan
CADASIL: cerebral autosomal dominant artheriopathy with subcortical infarcts and leukencephalopathy
CBV: cerebral blood volume
CGRP: calcitonin-gene related peptide
CI: confidence interval
CNS: central nervous system
Cr: creatine/phosphocreatine
CSD: cortical spreading depression
CSF: cerebrospinal fluid
CT: computed tomography
DWI: diffusion-weighted imaging
FDG: 2-deoxy-2\(^{18}\)F-fluoro-D-glucose
FHM: familial hemiplegic migraine
FLAIR: fluid-attenuated inversion recovery
T1-gad: gadolinium contrast enhanced T1-weighted MRI
IDO: indoleamine 2,3-dioxygenase
IDH: isocitrate dehydrogenase
MELAS: mitochondrial encephalopathy with lactic acidosis and stroke-like episodes
MGMT: methyltransferase
MRI: magnetic resonance imaging
MRS: magnetic resonance spectroscopy
MS: multiple sclerosis
NAA: N-acetyl-aspartate
NAWM: normal appearing white matter
PET: positron emission tomography
PTEN: phosphatase and tensin homolog
PWI: perfusion-weighted imaging
ROI: region of interest
RR: relative risk
SUV: standardized uptake value
TDO: tryptophan dioxygenase
TSH: thyroid stimulating hormone
WHO: World Health Organization
WML: white matter lesion
INTRODUCTION

This thesis is a summary of the research I’ve done in migraine and brain tumor neuroimaging. The first section will introduce the principles of neuroimaging and techniques pertinent to the thesis. The second section will cover migraine, whereas the third section will be about brain tumor research. Migraine is a very common disorder with very subtle pathophysiology and low mortality, whereas brain tumors are relatively rare, yet associated with drastic pathological changes and high mortality. This profound dissimilarity between the two diseases will be reflected in the structure of their designated sections.

My research on migraine revolved around the following questions: Are white matter abnormalities on neuroimaging associated with migraine? Do they bear morphological features specific to migraine? How do these abnormalities develop? Are these abnormalities limited to the locations revealed by conventional magnetic resonance imaging (MRI) or is the white matter globally affected in these patients? Therefore, due to the subtle nature of these changes, the pathophysiology of migraine will be described in detail. Factors that could affect our findings regarding the above questions will be pointed out.

My research on brain tumors focused on identification of information provided by alpha-[\(^{11}\)C]-methyl-L-tryptophan (AMT) positron emission tomography (PET) that could complement conventional MRI, and how this supplementary information could be utilized to improve outcomes for brain tumor patients. Therefore, the brain tumor section will discuss main differences between brain tumor types, describe parameters pertinent to survival, and how these factors may affect imaging.
MAGNETIC RESONANCE IMAGING

Magnetic resonance imaging (MRI) is based on magnetic manipulation of protons to acquire images without ionizing radiation. In an MRI scanner, the patient is placed in a powerful magnetic field, which aligns the magnetic moments of the protons in the patient’s body (Hashemi et al., 2004). A radio frequency electromagnetic field is switched on realigning the protons, and then switched off allowing the protons to return to their pre-excitation state (relaxation). The radiofrequency signals released during relaxation are detected by receiver coils and processed for subsequent image reconstruction. Spatial information is derived from 3-dimensional magnetic field gradients in which the position of different voxels is encoded by slightly different field strengths. During image reconstruction, various relaxation parameters can be weighted to attain contrast between distinct tissue types, which makes MRI a remarkably flexible diagnostic tool. Image weighting is most commonly based on either of the two principle relaxation parameters T1 or T2. T1 represents the time required for the longitudinal magnetization to recover ~63% of its pre-excitation value (spin-lattice relaxation), whereas T2 represents the time required for the transverse magnetization to decay to ~37% of its initial value (spin-spin relaxation). Conventionally, on the T1-weighted images the intensity (brightness) values assigned to voxels are visualized inversely proportional to the spin-lattice relaxation time (i.e. T1), therefore in normal brain tissue, black (hypointense) voxels represent the cerebrospinal fluid (CSF; T1 > 4000 ms), white (hyperintense) voxels represent white matter (T1≈780 ms), whereas the gray matter appears as gray (intermediate intensity; T1≈920) voxels. Since T1-weighted imaging describes normal anatomy with high fidelity, it is also called “anatomical”
imaging. In contrast, T2-weighting displays intensities directly proportional to the spin-spin relaxation time (i.e. T2), and thus visualizes increased tissue water content as hyperintensities, highlighting edema that is present in most pathological processes. Therefore, T2-weighted imaging is often called pathological imaging.

Conventionally, clinical MRI protocols comprise regular T1- and T2-weighted images, and also include a T1-gadolinium enhanced sequence (T1-gad) as well as a modified T2-weighted sequence called fluid-attenuated inversion recovery (FLAIR). The T1-gad image is generated following intravenous administration of a gadolinium-based contrast agent that enhances T1-weighted images by decreasing T1 relaxation time and thus highlight normal and abnormal vasculature as well as impaired blood-brain barrier integrity. In addition, FLAIR further increases the lesion-to-normal contrast of T2-weighed imaging by suppressing the CSF signal and therefore is an extremely sensitive measure of edema (De Coene et al., 1992; Hajnal et al., 1992; Rydberg et al., 1994). Patterns of changes visualized by conventional MRI can help identify pathological processes. For instance, a white matter lesion that is hypointense on the T1-weighted images and hyperintense on T2/FLAIR suggest the presence of a chronic process that includes severe structural damage and axonal loss within the lesion as seen with chronic infarcts, or multiple sclerosis (MS) plaques (van Walderveen et al., 1998), or even with brain tumors, especially when enhancing on T1-gad (Dhermain et al., 2010). Whereas, T2/FLAIR hyperintensity without hypointensity on T1-weighted images is a highly sensitive but non-specific marker of focal edema, which could be detected in relation to a developing MS plaque, or by tumor induced edema with or without tumor infiltration (Dhermain et al., 2010).

Advanced techniques may complement the information offered by conventional MRI. These techniques include diffusion-weighted imaging (DWI), perfusion-weighted imaging (PWI)
and MR spectroscopy (MRS) (Dhermain et al., 2010). DWI probes the Brownian motion of water molecules. The most commonly used DWI measure is the apparent diffusion coefficient (ADC) that is thought to reflect structural integrity as diffusion is limited by the presence of intra and extracellular structures within the brain. Bolus tracking following administration of a gadolinium contrast agent, allows estimation of cerebral blood volume (CBV) and blood flow (CBF), which can reveal focal or global impairments of brain perfusion that may be seen in relation to ischemic events, as well as identify regions with severe neovascularization and secondary capillary leakage, which is often observed in brain tumors. Finally, MRS enables voxel-wise quantification of metabolites such as the N-acetyl-aspartate (NAA) a putative neuronal marker, creatinine (Cr) that is present in both glial and neuronal cells, and lactate (Lac) that is thought to be an indicator of mitochondrial function (Aradi et al., 2013a). Levels of these MRS markers may be altered in the presence of tissue injury and/or brain tumor.

**POSITRON EMISSION TOMOGRAPHY**

Positron emission tomography (PET) is an imaging method that utilizes radioactive tracers (radiotracers) to image metabolism **in vivo**. PET is based on image reconstruction from data acquired via coincident detection of annihilation gamma photon pairs heading to (nearly) opposite directions. The annihilation photons are generated by a collision of an electron with a positron that is released during the beta-decay of a radionuclide. Since the radionuclide is located very close to the axis of the annihilation photons, detection of multiple photon pairs enables localization of the radionuclide in space. The radiotracer is made of a beta-decaying radionuclide, such as Fluorine-18 [18F], Carbon-11 [11C] or Oxygen-15 [15O] attached to a molecule metabolized via the pathway targeted for studying. Radionuclides are produced in
cyclotrons and subsequently added to the tracer molecule. The relatively long (~110 min) half-life of $^{18}\text{F}$ enables transportation of the radiotracers to even hundred miles of distance and thus utilization of $^{18}\text{F}$-based radiotracers for PET does not necessitate an on-site cyclotron. In contrast, due to its short half-life (~20 min), $^{11}\text{C}$-tracers cannot be transported, limiting the availability of these tracers to centers with on-site cyclotrons.

The radiotracer utilized for the research covered by this thesis was the alpha-$^{11}\text{C}$methyl-L-tryptophan (AMT), which was originally invented to visualize brain tryptophan metabolism in relation to serotonin synthesis (Diksic et al., 1990b; Nagahiro et al., 1990).
MIGRAINE

Migraine is a primary headache disorder that is characterized by recurrent unilateral throbbing head pain typically accompanied by nausea and/or vomiting, photo- or phonophobia and avoidance of routine physical activity (International Headache Society, 2013 and 2004). The majority of patients only experience the migraine headache and their disorder is classified as “migraine without aura” (formerly called common migraine or hemicrania simplex); whereas, in about one-third of the patients migraine headaches are preceded by focal neurological symptoms called an aura and are classified as migraineurs with aura (formerly named classic migraine or complicated migraine) (Ferrari, 1998; International Headache Society, 2013 and 2004). Migraine affects about 12% of the general population of North America and Europe and has a lifetime incidence of 43% in females and 18% in males (Stewart et al., 2008). It causes significant disability and decrease in quality of life (Lipton et al., 2000; Terwindt et al., 2000), resulting in a total of 112 million bed-ridden days yearly (Hu et al., 1999). As most migraine sufferers are in their most productive years, the economic impact of this is highly significant, with estimated indirect costs reaching $15 Billion in the United States, and €27 Billion in Europe (Andlin-Sobocki et al., 2005; Hu et al., 1999). Therefore, even small improvements in the disease could have a huge overall impact, making migraine a disease worth studying.
Etiology and Pathophysiology

The exact etiology of migraine is not yet precisely known; however, extensive research of the past century revealed a multifaceted pathophysiology with involvement of genetic factors (Cutrer & Smith, 2013; Edvinsson et al., 2012; Gasparini et al., 2013), intra and extracranial dysregulation of neuropeptides and monoamines and secondary sterile inflammation (Kaiser & Russo, 2013; Messlinger et al., 2011), as well as changes of brain circulation and alteration of pathways responsible for the regulation and processing of pain (Borsook & Hargreaves, 2010; Colombo et al., 2012; Goadsby, 2005; Schwedt & Dodick, 2009; Tedeschi et al., 2012).

The first and foremost question regarding migraine is “what is hurting actually?” This has been studied and answered by Ray and Wolf in 1940. In the course of neurosurgery, they queried locally anesthetized patients about their experience during electric stimulation of intra and extracranial structures of their heads (Ray & Wolff, 1940). According to their report, pain from the supratentorial structures is referred to the face (i.e. the region anterior to the ears) thus belongs to the afferentation of the trigeminal system, whereas infratentorial pain is referred to the back of the head. They found that most extracranial structures are pain-sensitive on the head, especially the arteries, whereas intracranially only venous structures, the dura and cerebral arteries at the base of the skull are sensitive to pain. Nonetheless, recent reports argue that sensitization and/or persistent stimulation of the dural and pial surface in migraine could expand pain sensitivity far beyond the above-described regions, and thus could at least in part explain the emergence of pain and sensitivity for head motion during the migraine headache (Olesen et al., 2009).

The next question is “what is happening during a migraine attack?” Historically, there have been two prevalent independent models to explain the occurrences in migraine: the vascular
and the neuronal theories (Gasparini et al., 2013; Tfelt-Hansen & Koehler, 2011). While neither is capable of explaining all migraine symptoms, the tremendous overlaps between the two models lead to their integration into the current neurovascular view of the disease (Edvinsson et al., 2012; Gasparini et al., 2013; Tfelt-Hansen & Koehler, 2011).

The vascular theory postulates that migraine is mediated by intracranial vasoconstriction followed by vasodilatation (Gasparini et al., 2013; Tfelt-Hansen & Koehler, 2011). This is supported by historical experimental data (Graham & Wolff, 1938), along with emerging neuroimaging data demonstrating cerebral blood flow changes associated with migraine (Borsook & Hargreaves, 2010; Colombo et al., 2012; Goadsby, 2005; Schwedt & Dodick, 2009; Tedeschi et al., 2012). Furthermore, a recent magnetic resonance angiography study found that vasodilation related to migraine pain is restricted to intracranial arteries and is lateralized to the side of the pain (Amin et al., 2013). This finding is not only corroborating the role of vasodilatation in the pathogenesis of migraine, but also places it as a mainly cerebrovascular disorder. Pre- or post-attack ischemia could possibly explain the development of white matter lesions in migraineurs, and would imply that appearance of these lesions is predisposed to the watershed zones, which are highly vulnerable to ischemia.

The vascular view is reinforced by the pharmacological observation that many of the abortive medications (experimental or clinically used) have a vasoconstrictive effect, such as ergotamine (Graham & Wolff, 1938), methysergide (Saxena, 1974), sumatripan (Amin et al., 2013; Humphrey et al., 1991), or at least reverse vasodilatation like the new CGRP antagonists olcegepant and telcagepant (Recober & Russo, 2007; Tepper & Cleves, 2009). There is also data available from the opposite direction, showing that nitroglycerine, is capable of provoking genuine migraine attacks in migraineurs (Thomsen et al., 1994) and bilateral throbbing
headaches in healthy non-migraineurs (Iversen et al., 1989) probably by triggering vasodilatation, which could also explain the pulsatory character of migraine pain. The vascular effect of the above-mentioned drugs also insinuates that pharmacological migraine therapy may also affect, maybe prevent the development of white matter lesions.

The neuronal theory postulates that migraine is triggered by a primary CNS event causing dysfunction of neuronal networks of the susceptible individual (Edvinsson et al., 2012; Gasparini et al., 2013). It may work operate through an abnormal trigeminal activation with release of neuropeptides that sensitize normally non-nociceptive structures resulting in neurogenic inflammation, vasodilatation, and the perception of pain as putative epiphenomena of the original event (Kaiser & Russo, 2013; Messlinger et al., 2011; Olesen et al., 2009). Altered brain functioning could explain phono- and photophobia, and has also been implicated in the development of the aura symptoms. This also suggests that migraine related structural damage is not restricted to the white matter changes. In the 1940s Lashley described the spreading rate and pattern of scotomata experienced during an aura (Lashley, 1941). Subsequently, Leão discovered the cortical spreading depression (CSD) in rabbits (Leo, 1944), which consists of marked decrease of electrical activity after focal electrical stimulation of the brain spreading slowly throughout the cortex in a shockwave-like manner. Although the link between CSD and the aura was proposed in 1958 (Milner, 1958), to date, there is only indirect evidence for the existence of CSD in humans with migraine (Charles & Brennan, 2009). Nevertheless, CSD is now regarded one of the main occurrences in migraine with aura, yet it still does not explain the 70% of migraine headaches that occur without an aura (Gasparini et al., 2013). Although the circulatory changes attributed to CSD could also play a role on the development of white matter changes as
well, it would also imply that white matter changes are exclusive, or more severe in migraineurs with aura, a notion that our research findings do not corroborate (Trauninger et al., 2011).

A significant genetic component in the migraine mechanism has long been presumed on basis of higher incidence of migraine among first-degree relatives of migraineurs (Lemos et al., 2009; Stewart et al., 1997). Inheritability has also been confirmed by multiple twin studies (Gervil et al., 1999; Larsson et al., 1995). In 1995, a Ca\(^{2+}\)-ion channel coding gene mutation was linked to a special migraine subtype called familial hemiplegic migraine (FHM) (Ophoff et al., 1996). Subsequently, two additional genes associated with FHM were discovered, both coding ion-channels (De Fusco et al., 2003; Dichgans et al., 2005). Besides drawing much attention to the genetics of primary headaches, these findings gave rise to the hypothesis of increased neuronal excitability promoting migraine due to abnormal ion-channels, which fit well into the neuronal theory (Gasparini et al., 2013). This hypothesis is supported by the discovery of the mutation of the KCNK18 gene that codes a K\(^{+}\)-channel linked to migraine with aura (Lafreniere et al., 2010). However, no similar mutation has yet been found for migraine without aura implying heterogeneous genetic background and non-Mendelian mode of inheritance (Gasparini et al., 2013). This variability could also explain the reproducibility issues of migraine related genetic findings, especially among populations of different ethnicities (Gasparini et al., 2013). This has been the case with the genetic polymorphisms of the SLC6A4 serotonin transport molecule that is responsible for the reuptake of serotonin from the synapses. The SLC6A4 gene related promoter polymorphisms (5-HTTLPR) were not found to be in overall association with migraine (Schurks et al., 2010a), whereas polymorphism of an intron of the same gene (STin2 VNTR 12/12) was indeed associated with migraine (Liu et al., 2011), especially in patients of European descent (Schurks et al., 2010b). Both of these are associated with slower serotonin
removal from the synapses. Such alterations of serotonin metabolism may be important in the microvascular changes in the white matter in migraines, considering serotonin is a potent vasoconstrictor (Tfelt-Hansen & Koehler, 2011) and serotonergic terminals are intimately associated with intracerebral small vessels, especially in the fronto-parietal cortex (Cohen et al., 1996; Cohen et al., 1995).

Other genes associated with microvascular damage were also identified in relation to migraine, such as the NOTCH3, or the methylenetetrahydrofolate reductase (MTHFR) genes (Gasparini et al., 2013). Mutation of the NOTCH3 is responsible for the development of the cerebral autosomal dominant artheriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) (Liem et al., 2010), in which severe degeneration of vascular smooth muscle occur and is associated with highly increased incidence of migraine with aura as well as white matter lesions (Liem et al., 2010). Certain mutations of the MTHFR gene are associated with increased serum homocysteine level, which accelerate atherosclerosis and therefore is a cardiovascular risk factor (Hofmann et al., 2001; Veeranna et al., 2011), and is also associated with predisposition to migraine, especially with the migraine with aura subtype (Kowa et al., 2000). Hence, we measured serum homocysteine levels in our study of risk for white matter abnormalities in migraine.

Finally, disturbance of the oxidative phosphorylation due to mitochondrial dysfunction has been implicated in the pathomechanism of migraine (Gasparini et al., 2013; Sparaco et al., 2006). Increased lactate in the brain of migraineurs as measured by magnetic resonance spectroscopy (MRS) (Watanabe et al., 1996) has been indicated previously, as well as an association between some mitochondrial diseases such as mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS) pointing towards the direction of
mitochondrial dysfunction (Sparaco et al., 2006). Therefore, in our advanced imaging study, we included MRS analyses of factors related to mitochondrial functioning such as Lactate (Lac), or the Creatinine/Phosphocreatinine (Cr) fraction and tested their changes within the white matter abnormalities as well as in normal appearing white matter.

**COMORBIDITIES AND OUTCOMES**

Migraine has been traditionally thought of as a psychosomatic disorder, and although there is mounting evidence about the biological determination of the disease, it is important to mention that migraine is associated with a high incidence of depression (17-26%) and anxiety (~19%) (Buse et al., 2010). Unsurprisingly, chronic pain is also common among migraineurs (Buse et al., 2010). A link between migraine and cerebrovascular and cardiovascular risk has also been discovered. According to a meta-analysis of 25 carefully selected studies (Schurks et al., 2009), the pooled relative risk (RR) associated with migraine was 1.73 for ischemic stroke (95% CI: 1.31-2.29), which was even higher if the migraineurs were female (RR 2.08), aged younger than 45 years (2.65), smokers (9.03), or were current users of oral contraceptives (7.02). Further analysis by Schürks et al. of studies that reported aura status indicated that migraine with aura is stronger linked to stroke (RR 2.16, 95% CI: 1.53-3.03). Female migraineurs with aura were also found to have heightened risk for myocardial angina and infarction (Kurth et al., 2006). While these studies revealed a clear link between migraine with aura and cerebro-/cardiovascular risk, the association of these diseases and migraine without aura is not clear. Although based on these data, one would assume such differences in aura status would manifest in some form in the incidence or severity of WMLs in migraine, we found such difference in none of our WML studies (Aradi et al., 2013b; Kamson et al., 2012; Trauninger et al., 2011).
An interesting notion of migraine-stroke comorbidity is that WMLs seen in migraineurs may be regarded as subclinical strokes. One of the proposed connections between the two diseases is repetitive paradoxical microembolisms via a patent foramen ovale (Carod-Artal et al., 2006; Lechat et al., 1988; Nozari et al., 2010). However, this notion has not been substantiated by a recent meta-analysis (Davis et al., 2013), neither was it associated with WMLs in our population study of migraineurs (Trauninger et al., 2011).

While the prevalence of tension type headaches is not increased in multiple sclerosis (MS), migraine is about three times more common among patients suffering from (MS) than in the general population (Kister et al., 2010; Truini et al., 2013). The clinical relevance of this connection is that MS may occasionally present with migraine as the first symptom (D'Amico et al., 2004; Freedman & Gray, 1989; Lin et al., 2013). Also, migraineurs may have radiographic presentation very similar to that of MS (Casini et al., 2013). Therefore, differentiation of WMLs secondary to migraine or MS could possibly facilitate the clinical distinction of migraineurs with and without MS.

Finally, since this thesis covers migraine and brain tumors, it should be noted that brain tumors can be associated with and present with headaches as well. However, most of the headaches that are associated with brain tumors are non-classifiable into any typical headache type and are predominantly associated with brain edema, increasing intracranial pressure and vomiting (Pfund et al., 1999).
SUMMARY OF MIGRAINE RESEARCH SUPPORTING THE THESIS

First, we studied the lab and imaging parameters of 186 carefully selected patients who visited our Neurology Clinic at the University of Pécs between 2007 and 2009 for migraine headaches (Trauninger et al., 2011). To avoid bias, we excluded patients with severe comorbidity, such as diabetes, hypertension, abnormal T₃/T₄ hormone levels, vasculitides, demyelinating disease, or genetically inherited syndromes (e.g. CADASIL). White matter lesion (WML) was defined as abnormal hyperintensity of FLAIR without hypointensity on T1-weighted images. We found WMLs in of 31% of the patients, and only 4 of them had lesions infratentorially. Attack frequency and disease duration were independent predictors of WML presence. In addition, higher incidence of WMLs was also associated with hyperhomocysteinemia, and subclinical thyroid dysfunction (i.e. TSH outside the normal range). Nevertheless, the incidence of WMLs did not differ between the migraine with and without aura groups. Next, we studied and compared the distribution of WMLs in migraineurs with multiple supratentorial lesions to an MS patient group (Kamson et al., 2012). Migraine WMLs were uniformly distributed and small within the lobes, mainly affecting the subcortical U-fibers and the deep white matter and chiefly belonged to the anterior circulation. In contrast, MS patients had larger WMLs and higher total lesion load. Although most likely insufficient to distinguish the two groups, the main difference between the groups was the smaller lesion size, count and load in the occipital and temporal lobes in migraineurs compared to the MS patients.

As the next step, we studied and compared the same group of migraineurs with WMLs to a group of healthy controls, utilizing advanced quantitative MRI methods including measurement of the apparent diffusion coefficient (ADC), T1- and T2-relaxation times, the concentration of
certain metabolites on MR spectroscopy (MRS) and the cerebral blood flow (CBF) and cerebral blood volume (CBV) (Aradi et al., 2013b). No difference was found between the normal appearing white matter (NAWM) of migraineurs and the white matter of the controls. On the other hand, the WMLs had increased ADC and T2-relaxation times suggestive of increased free water fraction that is likely to be secondary to tissue damage within the lesion. Furthermore, on MRS the N-acetyl-aspartate (NAA) as well as the Creatinine (Cr) fractions were decreased. Since NAA is a neuronal marker, its decrease may indicate axonal loss or demyelination, whereas Cr is a glial and mitochondrial marker, thus its decrease suggests either glial or mitochondrial dysfunction, or both.

In summary, WMLs are highly prevalent among migraineurs and are associated with important migraine metrics such as disease duration and attack frequency. They are very similar to the WMLs of MS patients, but smaller, uniform in size and relatively rare in the temporal and occipital lobes. The NAWM of migraineurs appears to have normal perfusion and metabolism, whereas signs of both glial/mitochondrial and neuronal damage are seen within the WMLs. Future directions of our research of migraineurs will be the longitudinal analysis of WMLs, and studying the cortex (e.g. cortical thickness) in relation to the presence or absence of WMLs.
PAPER I.

Volumetric comparisons of supratentorial white matter hyperintensities on FLAIR MRI in patients with migraine and multiple sclerosis


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(Kamson et al., 2012)

PROBLEM

- Multiple sclerosis causes white matter lesions
- Migraine is an independent risk factor of white matter lesions
- Migraine is very common in MS
- White matter lesions in migraineurs and MS patients appear similar

GOAL

- To find a difference in distribution or size between the lesions of the two patient groups that could be used to distinguish them from each other

APPROACH

- FLAIR MRI was acquired from patient with migraine and MS
- The white matter lesions were localized, counted and their volumes were measured

FINDINGS

- Migraine WMLs were uniformly distributed and small within the lobes and mainly affected the subcortical U-fibers and the deep white matter
- MS patients had larger WMLs and higher total lesion load
- Migraineurs had smaller lesion size, count and load in the occipital and temporal lobes compared to MS patients
- Migraine WMLs were distributed within the territories of the anterior circulation
Volumetric comparisons of supratentorial white matter hyperintensities on FLAIR MRI in patients with migraine and multiple sclerosis

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ABSTRACT

Migraine and multiple sclerosis (MS) can both cause white matter lesions that appear similar on conventional MRI. This study aimed to compare these abnormalities, and to find anatomical biomarkers specific for migraine. Supratentorial white matter hyperintensities (WMH) of 17 migraineurs and 15 patients with MS were counted, volumetrically analyzed, and their lobar distribution assessed on fluid-attenuated inversion recovery MRI. We found that migraine WMH affected mainly the deep white matter and subcortical U-fibers, belonged to the anterior circulation, appeared more frequently in the frontal and parietal lobes, showed no difference in average size between lobes, and were smaller and fewer than in MS. Most of the MS WMH were in the frontal lobe and were the smallest average size, while the fewest WMH with the largest size were in the occipital lobe. The pattern of supratentorial WMH appearance differs between the two groups; however, accurate differential diagnosis of WMH by conventional MRI is probably not possible in individual patients.

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1. Introduction

Migraine is a neurovascular disorder characterized by recurrent headache attacks, associated with temporary symptoms of autonomic nervous system dysfunction and, in some patients, attacks are accompanied by transient focal neurological aura.1 Based on MRI findings, migraine is an independent risk factor for supratentorial deep white matter lesions, silent posterior circulation territory infarcts, and infratentorial hypertensive lesions.2–5

Multiple sclerosis (MS) is one of the most common disorders of the central nervous system (CNS), characterized by inflammatory demyelination disseminated in different areas of the CNS and occurring at different times.6 Most patients have a relapsing course from onset and their attacks are associated with focal neurological symptoms.6

Supratentorial migraine-related white matter lesions are small, ovoid or circular with distinct borders, located in both the periventricular and deep brain white matter;2 and are seen as hyperintensities on T2-weighted and fluid-attenuated inversion-recovery (FLAIR) MRI without hypointensity on T1-weighted MRI.7 Supratentorial white matter lesions in MS are larger than those related to migraine, and are typically oriented perpendicular to the lateral ventricles with less distinctive borders, but can be juxtacortical, and subcortical, as well.8 Similar to migraine, white matter lesions in MS with acute or subacute inflammation and edema are best visualized on T2-weighted and FLAIR MRI, while lesions with severe axonal damage and glial necrosis are also visible on T1-weighted MRI.8

Multiple studies found that primary headaches – including migraine – have a higher prevalence in patients with MS, when compared to the general population.9–11 Most patients already have headaches before the onset of MS and some overlap in the pathophysiology of MS and migraine exists.10 These overlapping mechanisms in the pathophysiology may explain the similar radiological appearance of the white matter lesions on conventional MRI. Furthermore, when there is migraine–MS coexistence, it is not possible to differentiate the migraine-related lesions from the MS plaques by conventional MRI, particularly in those patients where the appearance of the lesions (size, shape, location, distribution, signal intensity) is not typical for either migraine or MS. The differentiation of migraine and MS-related lesions is important since newly formed white matter hyperintensities (WMH) may influence therapeutic decisions.

Thus, we aimed to count, localize and measure the volumes of supratentorial WMH of migraineurs and patients with MS using high-resolution FLAIR MRI to define the similarities and differences.
between the migraine and MS groups, as well as to find migraine-specific anatomical biomarkers.

2. Patients and methods

The research protocol was approved by the Regional Research Ethics Committee of the Medical Center, Pécs. Informed consent was given by all participants.

2.1. Participants

Seventeen patients with migraine were enrolled prospectively into the study (15 females and two males, mean age ± standard deviation [SD]: 42.6 ± 11.2 years; aged 19–67 years). Ten patients fulfilled the International Headache Society (IHS) classification criteria of migraine without aura and seven patients of migraine with aura. Migraineurs were chosen if they had supratentorial WMH on their T2-weighted and FLAIR MRI without hypointensity on T1-weighted MRI. No selected migraine patient had infratentorial signal abnormality. Further, selected patients lacked major comorbidities, including hypertension, cardiac disease, diabetes, thyroid gland dysfunction, oncological and hematological diseases, infectious diseases (such as human immunodeficiency virus [HIV], hepatitis), CNS demyelination (including MS), and genetically inherited disorders (such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy [CADASIL]). Migraine patients with other types of headaches were also excluded from the study. All measurements were taken in an interictal period and no patient was taking chronic migraine prophylactic treatment at the time of the MRI study.

Fifteen age-matched patients with MS with supra- and infratentorial WMH were prospectively enrolled in the study (11 females and 4 males, mean age ± SD: 37.3 ± 9.0 years; age range 23–51 years; compared to the age of the migraine group: unpaired t-tests, p > 0.05; mean Expanded Disability Status Scale score ± SD: 1.6 ± 1.53; EDSS range: 0–4). All patients were diagnosed with relapsing-remitting MS according to the 2005 modified McDonald criteria. All measurements were taken in the remission phase and the patients were on chronic immunomodulatory therapy at the time of the MRI study. Subjects having migraine headaches and cerebrovascular risk factors were excluded from the study. A further exclusion criterion was the presence of lesions apparent on T1-weighted MRI.

2.2. MRI protocol

MRI was performed on a 3.0-Tesla clinical MRI scanner (Magnetom TIM Trio, Siemens Medical Solutions, Erlangen, Germany) with a field gradient strength of 40 mT/m and a 12-channel phased array head coil. Beyond the routine T1 and T2 measurements and diffusion analysis, three-dimensional (3D) FLAIR studies were also performed. The 3D FLAIR MRI were acquired with turbo spin echo sequence (TR/TE: 15710/2750.8/105 ms; 100 slices; slice thickness: 1.5 mm; distance factor 0% [e.g. no gap], interleaved slice readout with two concatenation, FOV: 220×220 mm², 192×192 pixel matrix; bandwidth: 400 Hz/pixel; number of echo trains: 14). WMH was considered if visible as hyperintensity on T2-weighted and FLAIR MRI but without hypointensity on T1-weighted MRI and were larger than 3 mm, appearing in at least two consecutive slices.

2.3. Data analysis

The software evaluation tool for the images, 3D Slicer 2.6 was used. Supratentorial WMH were marked manually, pixel-by-pixel (1.099–1.15 mm), on all FLAIR MRI to create label maps (Fig. 1a–c). The volume of label maps was estimated using 3D Slicer’s MeasureVOL module. A 3D model was created of each lesion label map and of each patient’s gray matter to aid the differentiation of lobe boundaries and the location of lesions (Fig. 1d). Borders were defined by aligning orthogonal planes along the anatomical boundaries of the lobes: (i) frontal lobe: the central sulcus; lateral sulcus; (ii) parietal lobe: central sulcus; a horizontal plane elongating the lateral sulcus; a vertical plane between the parietooccipital sulcus and the parieto-occipital incisure; (iii) temporal lobe: lateral sulcus and a horizontal plane elongating the lateral sulcus; a vertical plane between the parietooccipital sulcus and the parietooccipital incisure; and (iv) occipital lobe: a vertical plane between the parietooccipital sulcus and the parietooccipital incisure.

WMH were counted, volumes were measured (mL/patient) and the average size of the individual WMH (mL per hyperintensity) was calculated for each patient. The results were categorized as the total of all lesions, and the sums of each left and right pair of lobes (frontal, parietal, temporal and occipital). Since migraine is a neurovascular disorder, migraine-related WMH were divided by the blood supply of their location into two categories: anterior circulatory territory (internal carotid artery) and posterior circulatory territory (vertebrobasilar – posterior cerebral artery) hyperintensities. The arterial territories of the identified WMH were determined using previously published maps.

2.4. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences version 15.0 (SPSS; Chicago, IL, USA). All data from the measurements and calculations were collected and arranged into datasets according to patient groups (migraine, MS) and their location (frontal, parietal, temporal, occipital lobe, and the total of all four). Migraine WMH were categorized as periventricular, callososeptal, subcortical (U-fiber), and deep white matter lesions. Shapiro-Wilk-tests were applied to all datasets to determine whether they had a normal distribution. Datasets from the same patient group and from the same location of the two-patient groups were then compared to each other one-by-one. Unpaired t-tests were used if both compared datasets had a normal distribution. Since different variances between migraine and MS WMH were assumed, Welch’s correction was applied to the unpaired t-tests when both normal datasets belonged to different patient groups. Mann-Whitney probes were used if any of the compared datasets did not show normality. Correlations between clinical and WMH data were not investigated due to the relatively small number of patients. P-values lower than 0.05 were considered significant.

3. Results

3.1. WMH location, number and volume of supratentorial lobes

There were no differences between the right and left hemispheres in the number of WMH, total hyperintensity volume, and average hyperintensity size, in migraine sufferers or in patients with MS.

Of 17 migraineurs, 16 patients had WMH in the frontal lobe, 16 in the parietal, seven in the temporal, and five in the occipital lobes. Of 15 patients with MS, all had WMH in the frontal lobe, 14 in the parietal lobe, 12 in the temporal lobe and 10 in the occipital lobe. In migraine sufferers, except for the WMH in the occipital lobe, all WMH were found in the anterior vascular territory, which contained 97.1% of the WMH, covering 97.2% of all WMH volume. Of seven migraine patients with aura, three had posterior territory WMH (42.9%), while only two patients out of 10 without aura had WMH in the same area (20%).
Although patients with MS tended to have a higher mean number (± SD) of WMH (24.5 ± 19.3 WMH/patient) compared to those with migraine (18.4 ± 12.3 WMH/patient) (Table 1, Fig. 2), patients with MS had a significantly higher mean (± SD) supratentorial WMH volume (10.484 ± 12.936 mL/patient) compared to those with migraine (2.722 ± 3.274 mL/patient; \( p = 0.024 \)) (Table 1, Fig. 3). The volume of WMH ranged between 0.011 mL and 3.32 mL in migraine sufferers (mean [± SD] 0.177 ± 0.274 mL/WMH), which was smaller than those in patients with MS (range, 0.011–5.82 mL; mean, 0.402 ± 0.255 mL/WMH; \( p = 0.0041 \)) (Table 1, Fig. 4).

### 3.2. Mean lobar number of WMH

In the migraine group, more WMH were found in the frontal lobe compared to the parietal (\( p = 0.0007 \)), temporal (\( p < 0.0001 \)), and occipital (\( p < 0.0001 \)) lobes (Table 2, Fig. 2). On average more WMH were detected in the parietal lobe compared to the temporal (\( p = 0.0003 \)) and occipital (\( p < 0.0001 \)) lobes (Table 2, Fig. 2). No significant difference was found between the mean number of WMH in the temporal and occipital lobes (Table 2, Fig. 2).

On average, the MS group had more WMH in the frontal than in the temporal (\( p = 0.0013 \)) and occipital (\( p < 0.0001 \)) lobes, while the parietal lobe contained significantly more WMH compared to the occipital lobe (\( p = 0.0057 \)) (Table 2, Fig. 2). The mean number of WMH tended to be higher in the frontal lobe than in the parietal lobe, and in the parietal lobe than the temporal lobe (\( p = 0.064 \); \( p = 0.095 \); respectively). No statistical difference in mean number of WMH was found between the occipital and temporal lobes (Table 2, Fig. 2).

With respect to specific lobes, patients with MS had more WMH in the temporal lobe (\( p = 0.0082 \)) and occipital lobes (\( p = 0.048 \)) compared to patients with migraine (Table 2, Fig. 2).

### Table 1

Differences of the mean supratentorial white matter hyperintensity (WMH) number, mean hyperintensity volume (mL) and mean hyperintensity size (mL/hyperintensity) in the different hemispheric lobes and the totals of all lobes between migraine and multiple sclerosis (MS) patients

<table>
<thead>
<tr>
<th>Category</th>
<th>Group</th>
<th>Hyperintensity count ±SD</th>
<th>p-value</th>
<th>WMH volume (mL) ±SD</th>
<th>p-value</th>
<th>Volume/WMH (mL/hyperintensity) ±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>Migraine</td>
<td>18.4 ± 12.3</td>
<td>n.s.</td>
<td>2.722 ± 3.274</td>
<td>0.024</td>
<td>0.177 ± 0.274</td>
<td>0.0041</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>24.5 ± 19.3</td>
<td></td>
<td>10.484 ± 12.936</td>
<td></td>
<td>0.402 ± 0.255</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal lobe</td>
<td>Migraine</td>
<td>13.1 ± 8.8</td>
<td>n.s.</td>
<td>1.813 ± 2.393</td>
<td>n.s.</td>
<td>0.118 ± 0.140</td>
<td>0.0096</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>13.5 ± 10.8</td>
<td></td>
<td>3.606 ± 3.370</td>
<td></td>
<td>0.267 ± 0.235</td>
<td></td>
</tr>
<tr>
<td>Parietal lobe</td>
<td>Migraine</td>
<td>4.0 ± 3.4</td>
<td>n.s.</td>
<td>0.734 ± 1.142</td>
<td>0.022</td>
<td>0.184 ± 0.257</td>
<td>0.0065</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>6.3 ± 6.3</td>
<td></td>
<td>3.535 ± 6.123</td>
<td></td>
<td>0.543 ± 0.546</td>
<td></td>
</tr>
<tr>
<td>Temporal lobe</td>
<td>Migraine</td>
<td>0.8 ± 1.5</td>
<td>0.0082</td>
<td>0.099 ± 0.176</td>
<td>0.0059</td>
<td>0.141 ± 0.128</td>
<td>n.s. (0.091)</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>3 ± 3.0</td>
<td></td>
<td>2.068 ± 2.826</td>
<td></td>
<td>0.592 ± 0.608</td>
<td></td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>Migraine</td>
<td>0.5 ± 0.9</td>
<td>0.048</td>
<td>0.077 ± 0.151</td>
<td>0.0061</td>
<td>0.135 ± 0.082</td>
<td>0.0064</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>1.7 ± 2.0</td>
<td></td>
<td>1.275 ± 1.709</td>
<td></td>
<td>0.833 ± 0.615</td>
<td></td>
</tr>
</tbody>
</table>

A p-value less than 0.05 is considered significant, p-values between 0.05 and 0.1 are shown in italics.

n.s. = not significant, SD = standard deviation.

Passed the Shapiro-Wilk normality test.

Welch's corrected T-probe applied.
In patients with migraine, the mean lobar WMH volume was higher in the frontal than in the temporal (p < 0.0001) and occipital (p < 0.0001) lobes (Table 2, Fig. 3). Similar to the frontal lobe, a significantly greater mean WMH volume was detected in the parietal lobe than in the temporal (p = 0.0029) and occipital (p = 0.0005) lobes (Table 2, Fig. 3). The frontal lobe tended to have a higher average WMH volume than the parietal lobe (p = 0.076). No statistical difference was found between the temporal and occipital lobes (Table 2, Fig. 3).

In patients with MS, the mean frontal lobe WMH volume was greater than that of the occipital lobe (Table 2, Fig. 3: p = 0.020). The volume of the frontal lobe tended to be greater than the temporal lobe (p = 0.056). No other statistical significance was detected in the MS group (Table 2, Fig. 3).

When comparing patients with MS to those with migraine, the mean WMH volume was significantly larger in the MS group’s parietal (p = 0.022), temporal (p = 0.0059), and occipital (p = 0.0061) lobes, while the volume difference between the frontal lobes only showed a trend towards significance (p = 0.082) (Table 2, Fig. 3).

### 3.3. Mean lobar WMH volume (mL/lobe)

In patients with migraine, there was no statistical difference between lobes in mean WMH size (Table 2, Fig. 4).

In the patients with MS, the average WMH size in the occipital lobe was larger than in the frontal lobe (p = 0.020), and the WMH in the parietal lobe tended to be larger than in the frontal lobe (p = 0.099). There was no other statistical differences between the lobes (Table 2, Fig. 4).

When patients with MS and migraine were compared, the mean WMH size was significantly larger in the MS group in the frontal (p = 0.0096), parietal (p = 0.0065) and occipital (p = 0.0064) lobes, and there was a similar tendency between the temporal lobes (p = 0.091) (Table 2, Fig. 4).

### 3.4. Mean lobar WMH size (mL/hyperintensity)

In patients with migraine, there was no statistical difference between lobes in mean WMH size (Table 2, Fig. 4).

In the patients with MS, the average WMH size in the occipital lobe was larger than in the frontal lobe (p = 0.020), and the WMH in the parietal lobe tended to be larger than in the frontal lobe (p = 0.099). There was no other statistical differences between the lobes (Table 2, Fig. 4).

When patients with MS and migraine were compared, the mean WMH size was significantly larger in the MS group in the frontal (p = 0.0096), parietal (p = 0.0065) and occipital (p = 0.0064) lobes, and there was a similar tendency between the temporal lobes (p = 0.091) (Table 2, Fig. 4).

### 3.5. Migraine WMH in the periventricular, callososeptal, subcortical U-fiber and deep white matter

Migraine-related WMH were investigated in the characteristic MS locations: 13 periventricular WMH were found in six patients (35.3%); 17 callososeptal WMH were detected in 10 patients (58.8%); and 124 WMH were found with subcortical U-fiber involvement in 15 patients (88.2%). The remaining white matter abnormalities were located in the deep white matter (n = 158) and only one patient lacked WMH in this location (94.1%). More deep white matter and U-fiber abnormalities were found than callososeptal and periventricular WMH (p < 0.0005 in all cases).

### 4. Discussion

We investigated the migraine- and MS-related supratentorial WMH detected on high resolution FLAIR MRI using the 3D Slicer software evaluation tool. In these comorbid disorders, the individual lesions can have a similar radiological appearance; therefore, the differences in WMH number, volume and lobar distribution could be helpful in finding anatomical biomarkers.

In general, in patients with migraine, fewer and smaller WMH were detected than in patients with MS affecting the subcortical U-fibers and deep white matter more frequently than the periventricular and callososeptal regions. Both populations had the highest number of WMH and total WMH volume in the frontal and parietal lobes. In addition, the involvement of the tempo-occipital region proved to be more frequent in patients with MS than in
migraineurs. To some extent, the lobar distribution of WMH can be explained by the anatomical proportions of the cerebral lobes: the frontal lobe, which is the largest, has the highest probability of containing WMH. Thus, we calculated the average size of individual WMH, which equalized the differences between the lobes in the migraine group, whereas in patients with MS, the average WMH size was larger in the occipital than in the frontal lobe.

The differences between migraine and MS groups and the lobar differences in migraine can be based on a different pathophysiology of WMH formation. The pathogenesis of these migraine-related WMH is very likely multifactorial, since several pathophysiological mechanisms have been proposed, including attack-related oligemia and focal hypoperfusion. Repeated or prolonged reduced perfusion, sluggish cerebral blood flow and oligemia in large and/or small arteries have been described in migraine attacks. Reductions of cerebral blood flow vary from a 7% to a 53% decrease and persist from one hour to more than one day. This is probably the result of the effects of cortical spreading depression present in all migraine phenotypes, which directly alters the blood–brain barrier’s permeability, and therefore can lead to local cellular injury caused by ischemia with glutamatergic excitotoxicity and intracellular calcium-mediated apoptosis. The local release of vasoactive neuropeptides could result in further changes in cerebral hemodynamics. Interestingly, we identified most of the hemispheric WMH to be in the territory of the internal carotid artery circulation. The results of a previous study showed that the subclinical posterior territory infarcts and infarct-like lesions were mainly located in the cerebellum andpons, but not in the occipital and temporal lobes. Despite growing evidence that migraine attack affects the whole brain, there are regional differences and predilection sites for tissue damage. Although we cannot explain why the anterior circulation territory was more impaired than the posterior territory, we speculate that the underlying mechanism leading to WMH formation may be similar to ischemic stroke with lower overall prevalence of posterior territory hemispheric infarcts. We found that posterior territory WMH are more frequent in patients with aura than in patients without aura. Because of the small size of the migraine subgroups, it is hard to determine whether this finding was a coincidence or the result of a different pathomechanism (such as cortical spreading depression in migraine with aura that affects the posterior circulation territory more severely).

Conversely, the larger WMH sizes in all hemispheric lobes and the more disseminated lobar involvement in MS can be explained by a different undergoing pathologic process. Although a precise mechanism leading to plaque formation is not completely understood, disruption of the blood–brain barrier is a crucial step in the evolution of the MS lesion. The blood–brain barrier disruption can be initiated by autoreactive CD4+ lymphocytes that migrate into the CNS and initiate an inflammatory response. Upregulation of adhesion molecules on capillary endothelial cells, perivascular inflammation, and other factors can facilitate the invasion of leucocytes into the CNS. The inflammatory cascades result in oligodendrocyte death, axonal and neuronal toxicity, and via the sequestered CNS antigens, further cycles of autoimmune-induced inflammation is initiated. Similar to migraine, the vascular/ischemic basis of demyelination has been examined, but it is more probable that the observed reduction in cerebral blood flow and cerebral blood volume is secondary to immunologic processes.

In summary, in migraine, the supratentorial WMH appeared most frequently in the deep white matter and subcortically in the anterior circulation territory, mainly in the frontal and parietal lobes with very similar WMH sizes in all hemispheric lobes. In MS, there were a higher number of and larger WMH than in migraine, and the dissemination of plaques was more widespread throughout the hemispheric lobes. Although we found differences between the migraine and MS groups, the accurate differential diagnosis of these supratentorial WMH in individual patients by conventional MRI techniques is not possible.

Table 2
Mean number of supratentorial white matter hyperintensity (WMH) lesions, WMH volume (mL) and WMH size (mL/hyperintensity) of the different hemispheric lobes of patients with migraine and of those with multiple sclerosis (MS)

<table>
<thead>
<tr>
<th>Group and comparison</th>
<th>Lobe</th>
<th>No. WMH lesions p-value</th>
<th>Volume of WMH lesions p-value</th>
<th>Volume/WMH lesion p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migraine Frontal lobe</td>
<td>Migraine parietal</td>
<td>0.0007</td>
<td>n.s. (0.076)</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>Migraine temporal</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>Migraine occipital</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>n.s.</td>
</tr>
<tr>
<td>Migraine Parietal lobe</td>
<td>Migraine frontal</td>
<td>0.0007</td>
<td>n.s. (0.076)</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>Migraine temporal</td>
<td>0.0003</td>
<td>0.0029</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>Migraine occipital</td>
<td>&lt;0.0001</td>
<td>0.0005</td>
<td>n.s.</td>
</tr>
<tr>
<td>Migraine Temporal lobe</td>
<td>Migraine frontal</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>Migraine parietal</td>
<td>0.0003</td>
<td>0.0029</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>Migraine occipital</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Migraine Occipital lobe</td>
<td>Migraine frontal</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>Migraine parietal</td>
<td>&lt;0.0001</td>
<td>0.0005</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>Migraine temporal</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>MS Frontal lobe</td>
<td>MS parietal</td>
<td>n.s. (0.064)</td>
<td>n.s.</td>
<td>n.s. (0.099)</td>
</tr>
<tr>
<td></td>
<td>MS temporal</td>
<td>0.0013</td>
<td>0.0056</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>MS occipital</td>
<td>&lt;0.0001</td>
<td>0.020</td>
<td>0.020</td>
</tr>
<tr>
<td>MS Parietal lobe</td>
<td>MS frontal</td>
<td>n.s. (0.064)</td>
<td>n.s.</td>
<td>n.s. (0.099)</td>
</tr>
<tr>
<td></td>
<td>MS temporal</td>
<td>n.s. (0.095)</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>MS occipital</td>
<td>0.0057</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>MS Temporal lobe</td>
<td>MS frontal</td>
<td>0.0013</td>
<td>n.s. (0.056)</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>MS parietal</td>
<td>n.s. (0.095)</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>MS occipital</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>MS Occipital lobe</td>
<td>MS frontal</td>
<td>&lt;0.0001</td>
<td>0.020</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>MS parietal</td>
<td>0.0057</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>MS temporal</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

A p-value < 0.05 is considered significant, p-values between 0.05 and 0.1 are shown in italics. n.s. = not significant.

1 Welch’s corrected unpaired T-probe applied.
Acknowledgement

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References

BRAIN TUMORS

From an epidemiologic standpoint neoplasms of the brain can be considered the opposite of migraine. While migraine has colossal prevalence but very limited mortality in the general population (Schurks et al., 2011), brain tumors are relatively rare yet cause very high mortality (Dolecek et al., 2012). This is well demonstrated by the estimated costs involved in the treatment of these diseases (Olesen et al., 2012): in 2010, migraine affected 49,900,000 people in Europe, generating an overall healthcare cost of €18,463,000,000, but a per patient cost of only €370; in contrast, brain tumors affected 200,000 Europeans with an overall cost of €5,174,000,000, yet per patient costs were €21,590 on average. The greatest part of brain tumor related expenses covers standard treatment with surgical resection with concomitant radiation and chemotherapy (chemoradiation) (Stupp et al., 2005). Also, since neuroimaging plays an important role in the diagnosis, treatment planning and follow-up in brain tumors, the usage of novel and expensive neuroimaging techniques is also well justified in this disease. This demand for innovation makes neuroradiology of neoplastic disease an exciting field where advanced neuroimaging can go beyond the scope of research and enter the clinical realm.
NEOPLASMS OF THE BRAIN

Brain neoplasms are abnormal tissue masses caused by uncontrolled cell proliferation within the brain or in its direct vicinity. The age-adjusted annual incidence of primary brain tumors is approximately 21/100,000, 7 of which are tumors of the meninges (meningiomas) and 6 are glial neoplasms (gliomas) (Dolecek et al., 2012; Ostrom et al., 2013). The majority of gliomas are originated from astrocytes, such as diffuse and anaplastic astrocytoma subtypes, as well as glioblastomas that comprise more than half of all gliomas and 80% of all malignant primary brain tumors (Dolecek et al., 2012; Louis et al., 2007; Ostrom et al., 2013). Oligodendrogial and mixed oligoastrocytic tumors represent a smaller subgroup, taking up about 3% of gliomas. Although most likely underestimated, the annual incidence of metastatic brain tumors is approximately 10/100,000 although these numbers are most likely underestimated (Barnholtz-Sloan et al., 2004; Walker et al., 1985). About 15 to 20% of lung cancer metastasized to the brain, neoplasms of the breast and kidney and melanomas also have great propensity to invade the brain (Barnholtz-Sloan et al., 2004; Gavrilovic & Posner, 2005; Schouten et al., 2002).

Irrespective of whether they originate from the intracranial cavity as primaries or arrive there as metastases, neoplasms of the brain share a special habitat that distinguishes them from extracranial tumors. One of the unique features of this habitat is the constant volume of the intracranial space, which is not capable of expansion (except in infants per se), therefore growth of one intracranial compartment may only occur at the expense of another as described by the Monro-Kellie principle (Mokri, 2001). Consequently, the first presentation of intracranial neoplasms is often in the form of life threatening symptoms secondary to increased pressure on the brain or on its vascular supply. The other special feature of this habitat is the supportive role
of non-neoplastic glial cells in brain tumor invasion by sheltering and facilitating proliferation of cancer cells (Fitzgerald et al., 2008; Lorger & Felding-Habermann, 2010; Marchetti et al., 2000). In the light of these information, it is not surprising that in extracranial cancer the appearance of a brain metastasis is one of the worst possible events in terms of prognosis, which is also reflected by the TNM staging system (Edge & American Joint Committee on Cancer., 2010; Louis et al., 2007; NCI, 2013). Since this scenario is the starting point for primary brain tumors, the World Health Organization (WHO) histopathological grading system is more appropriate than the TNM for prediction of biological behavior and consequent choice of therapies for these tumors (Louis et al., 2007).

**Grading in Primary Brain Tumors**

Rather than strict histological classification, WHO grading provides a malignancy scale spans across a variety of tumors. The scale ranges from I to IV, on which grades I and II are considered low-grade, whereas grade III and IV are high-grade (see Table 1) (Louis et al., 2007). WHO grade I represents tumors with low proliferative potential that may be cured by surgical resection. Although grade II neoplasms are infiltrative in nature (e.g. hence the name infiltrative astrocytoma), they have low proliferative activity and are typically associated with longer than 5 years of survival. However, they have a tendency to recur and transform into higher grade. Grade III brain tumors (e.g. anaplastic astrocytoma) present with nuclear atypia and high proliferative rate with survival typically 2 to 3 years despite the administration of adjuvant chemotherapy and/or radiation (Stupp et al., 2005). Finally, grade IV tumors such as glioblastoma are highly
proliferative, have a tendency for necrosis and are associated with rapid disease evolution and fatal outcomes within a year (Louis et al., 2007).

Although WHO grading is the current mainstay for determining treatment and predicting outcomes, increasing numbers of molecular markers are discovered that can further refine brain tumor diagnosis and grading. Such markers are changes in O\textsuperscript{6}-methyl-guanine-DNA methyltransferase promoter (MGMT) activation status, presence of 1p/19q alteration, mutation of the isocitrate dehydrogenase (IDH1) gene, inactivation of phosphatase and tensin homolog (PTEN) gene or activation of the immunosuppressive kynurenine pathway (Ahmed et al., 2014; Mitsuka et al., 2013; Munn & Mellor, 2007; Uyttenhove et al., 2003; Wainwright et al., 2012). MGMT is an enzyme that removes DNA adducts generated by alkylating agents rendering both low- and high-grade glioma cells resistant to alkylating chemotherapeutic drugs (Esteller et al., 2000; Everhard et al., 2006; Hegi et al., 2005). Inactivation of the MGMT gene through methylation of its promoter eliminates this mechanism and therefore improves therapy response and thus survival. The fusion, or deletion of 1p/19q is a marker associated with more favorable prognosis that is most common in oligodendrogliomas but may be present in oligoastrocytomas as well as in pure astrocytomas (Ahmed et al., 2014; Kaloshi et al., 2007). The exact mechanism of how it improves survival is not clear, however it has been shown that 1p/19q deletion status is strongly associated with certain methylation patterns (Kaloshi et al., 2007). IDH is not part of the traditional oncogenic pathway and its role in glioma survival is also unconventional with an underlying mechanism not entirely understood (Kloosterhof et al., 2011). One of the proposed hypotheses is that the IDH1 phenotype has increased production of 2-hydroxyglutarate, which inhibits dioxygenases leading to altered DNA methylation (Ahmed et al., 2014; Ichimura, 2012). Nevertheless, the presence of IDH1 gene mutation is associated with more favorable survival in
grade II and III, as well as with secondary glioblastomas, whereas it is never present in primary glioblastomas (Ichimura, 2012; Kloosterhof et al., 2011; Sanson et al., 2009). In addition, PTEN activation may also help distinguish secondary glioblastomas from the prognostically less favorable group of primary glioblastomas (Ahmed et al., 2014). Finally, activation of the kynurenine pathway through the upregulation of indoleamine 2,3 dyogenicase (IDO) has also been associated with worse survival in gliomas through the evasion of antitumor immune response, a mechanism AMT-PET is intended to harness for imaging (Alkonyi et al., 2012a; Kamson et al., 2014; Mitsuka et al., 2013; Munn & Mellor, 2007; Uyttenhove et al., 2003; Wainwright et al., 2012). Discovery of the above listed markers does not only introduce targets for potential new drugs, but is also a huge step towards molecular grading achieved through tumor profiling, which could allow individualized treatment, better outcomes and more accurate prognostication for brain tumor patients.

Table 1. WHO grading of select primary brain tumors*

<table>
<thead>
<tr>
<th>Tumor group</th>
<th>WHO Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Astrocytic tumors</strong></td>
<td>I II III IV</td>
</tr>
<tr>
<td>Subependymal giant cell astrocytoma (SEGA)</td>
<td>✓</td>
</tr>
<tr>
<td>Pilocytic astrocytoma</td>
<td>✓</td>
</tr>
<tr>
<td>Diffuse astrocytoma</td>
<td>✓</td>
</tr>
<tr>
<td>Anaplastic astrocytoma</td>
<td>✓</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>✓</td>
</tr>
<tr>
<td>Gliosarcoma</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Oligodendrogial tumors</strong></td>
<td>I II III IV</td>
</tr>
<tr>
<td>Oligodendroglioma</td>
<td>✓</td>
</tr>
<tr>
<td>Anaplastic oligodendroglioma</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Oligoastrocytic (mixed) tumors</strong></td>
<td>I II III IV</td>
</tr>
<tr>
<td>Oligoastrocytoma</td>
<td>✓</td>
</tr>
<tr>
<td>Anaplastic oligoastrocytoma</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Meningeal tumors</strong></td>
<td>I II III IV</td>
</tr>
<tr>
<td>Meningioma</td>
<td>✓</td>
</tr>
<tr>
<td>Atypical meningioma</td>
<td>✓</td>
</tr>
<tr>
<td>Anaplastic / malignant meningioma</td>
<td>✓</td>
</tr>
</tbody>
</table>

*reproduced from (Louis et al., 2007)
CONVENTIONAL IMAGING IN BRAIN TUMORS

As described in the neuroimaging section, T1-gad, and T2/FLAIR sequences are the mainstays of conventional imaging of brain neoplasms (Ahmed et al., 2014; Dhermain et al., 2010). Most high-grade gliomas as well as metastases are visualized as gadolinium-enhancing masses on T1-gad. Enhancement develops in areas where the integrity of the blood brain barrier is compromised (Graif & Steiner, 1986; Whelan et al., 1987) and where microvascular permeability is increased (Vaquero et al., 2002), which is commonly seen in tumors with intense neovascularization and is a correlate of tumor grade (Assimakopoulou et al., 1997; Kracht et al., 2003). However, the size of enhancement highly underestimates the infiltrative tumor volume, and one-third of malignant brain tumors show no enhancement at all (Scott et al., 2002).

Although T2 and FLAIR hyperintensity is a highly sensitive marker of edema and is often used as a surrogate marker for non-enhancing tumor (Pope et al., 2011), it cannot differentiate purely vasogenic from tumor-infiltrated edema as demonstrated on edema caused by non-infiltrative meningiomas and thus overestimates the tumor volume (Bitzer et al., 1997). As a consequence of the above-mentioned, conventional MRI is not capable of accurate estimation of tumor volume, which is crucial for achievement of optimal resection and radiation field coverage, both important factors of in terms of outcome (Chaichana et al., 2014; Liang et al., 1991). In addition, recurrent both tumor and treatment-induced changes (e.g. necrosis) appear as increased contrast enhancement on T1-gad and as hyperintensity of T2/FLAIR, making assessment of response to therapy and prognostication very difficult with the use of conventional MRI (Pope et al., 2011; Quant & Wen, 2011). This is further complicated by the advent of anti-angiogenic tumor therapy, which may mask the recurrent or residual tumor by decreasing the enhancing and
T2/FLAIR hyperintense volume (Pope et al., 2011). Finally, conventional MRI does not provide direct information regarding the metabolic activity of tumors and thus beyond markers such as the presence of necrosis or excessive edema; it does not allow differentiation of histological tumor types.

Advanced MRI, such as such as MRS, DWI, PWI are capable of overcoming some of the above-mentioned limitations, and may detect infiltrating and anaplastic tumor beyond the enhancing volume (Dhermain et al., 2010). Level of infiltration can also provide some information about tumor grade. MRS may also help distinguish tumor types based on their spectroscopic profile, however, except from DWI that is also limited, in their present forms none of these modalities provide volumetric information that could be used for surgical and radiation planning directly. Aminoacid PET, including AMT-PET can be used to acquire volumetric information that can complement both conventional and advanced MRI and potentially improve outcomes in brain tumors (Juhász et al., 2014).
AMT-PET – FROM SEROTONIN SYNTHESIS TO BRAIN TUMORS

The alpha-[\textsuperscript{11}C]-methyl-L-tryptophan radiotracer was originally invented to quantify serotonin synthesis \textit{in vivo} (Chugani et al., 1998b; Diksic et al., 1990a; Diksic et al., 1990b; Muzik et al., 1997; Nagahiro et al., 1990). Ever since, a great number of AMT-PET papers have been published on serotonin and tryptophan metabolism in healthy subjects (Chugani et al., 1998b; Muzik et al., 1997; Nishizawa et al., 1998; Okazawa et al., 2000), as well as in psychiatric disorders such as depression (Berney et al., 2008; Frey et al., 2010), alcoholism (Nishikawa et al., 2009), as well as in autism (Chandana et al., 2005; Chugani et al., 1999a; Chugani et al., 1997). Alterations of AMT uptake have also been found in migraine supporting involvement of the serotonergic system in the disease (Chugani et al., 1999b; Sakai et al., 2008; Sakai et al., 2014).

The attention of AMT studies was drawn towards lesional brain disorders when increased AMT uptake was found in epileptic lesions such as epileptogenic tubers of tuberous sclerosis patients (Chugani et al., 1998a; Fedi et al., 2003; Juhász et al., 2014). This has been attributed to increased metabolism of AMT by indolealmine 2,3-dioxygenase (IDO), the rate-limiting enzyme of the kynurenine pathway (Chugani & Muzik, 2000; Yamazaki et al., 1985). The downstream products of which, such as quinolonic acid, have long been implicated in epileptogenesis (Lapin, 1978; Nakano et al., 1993). The role of IDO and the kynurenine pathway has also been linked to immunosuppression and consequent evasion of anti-tumor immune response in tumors (Munn & Mellor, 2007; Uyttenhove et al., 2003). Initial brain tumor studies did find substantial AMT uptake in both extracranial (in lung and breast cancer) (Juhász et al., 2009; Juhász et al., 2012b), and intracranial tumors (Alkonyi et al., 2012b; Juhász et al., 2006; Juhász et al., 2011) that are linked with expression of IDO (Juhász et al., 2014). The association of IDO expression and
tumors with increased AMT uptake has been confirmed by preliminary histopathological studies (Batista et al., 2009; Juhász et al., 2012b; Zitron et al., 2013). Finally, AMT-PET was found to be able to distinguish radiation injury from recurrent tumor in previously treated glioblastomas (Alkonyi et al., 2012a), suggesting that tryptophan metabolism changes can be harnessed by AMT-PET to acquire clinically relevant information.
SUMMARY OF BRAIN TUMOR RESEARCH SUPPORTING THE THESIS

My involvement with AMT-PET started with volumetric analyses of gliomas. While accurate assessment of glioma volume cannot be achieved via conventional MRI due to the infiltrative nature of these tumors, optimal tumor delineation is crucial for therapy planning, as the totality of initial surgical resection and subsequent radiation therapy are strong determinants of survival. On conventional MRI, T1-gad underestimates tumor volume, whereas T2/FLAIR tumor volume includes tissue with non-infiltrated edema. Therefore there is great need for a modality that is capable of accurate assessment of tumor volume and infiltration. For this reason, we studied AMT-PET, T1-gad, and T2/FLAIR defined tumor volumes in grade II-IV gliomas and assessed spatial concordance and discordance between the modalities (Kamson et al., 2013b; Kamson et al., 2012c; Mittal et al., 2012). Tumor presence was histologically verified in concordant and discordant regions in a group of patients. We found AMT-defined volumes to be larger than the T1-gad but smaller than the T2/FLAIR volume. AMT volume was discordant with MRI and correlated positively with the tumor proliferative index measured by histopathology. All tissue samples contained tumor cells within but not outside the AMT-defined volume. Tumor cell concentration was highest in samples from the sites of concordant AMT-T1-gad volume. These results support that AMT-PET is capable of accurate assessment of tumor volume that could optimize tumor therapy. In a subsequent study, we tested this notion by analyzing the patterns of radiation therapy failure (i.e. tumor recurrence) in patients with high-grade glioma (Christensen et al., 2013; Christensen et al., 2012). We found MRI- and AMT-defined radiation fields to have similar volumes, however, the coverage of initial recurrence volume was superior with AMT-PET (52% vs 68%) and even better when the AMT and MRI volumes were combined (73%). An extended combined AMT-MRI-based tumor volume would
have completely covered the initial progression in 71% of the cases. These results do support the possible usefulness of AMT-PET for pretreatment tumor delineation.

Our next project included analyses of metastatic brain tumors with the intent of identifying markers that could distinguish them from glioblastomas (Juhász et al., 2012a; Kamson et al., 2013c). The clinical relevance of this is that 15% of solitary brain metastases present without a known a primary tumor source, mimicking gliomas, therefore correct identification of the tumor source before surgery can alter the course of treatment and thus may improve outcome in these cases. The other relevance of this study is that, to investigate whether metastases in the brain retain some of the features of tumors from their primary sources. This is especially an interesting question, since as described in the text above, metastatic brain cancer is sheltered by glial cells and thus may acquire similar features to primary brain tumors. We found that with the use of AMT kinetic analysis, glioblastomas and metastatic brain tumors can be differentiated, and with the combination of MRI and AMT the accuracy for this can reach up to 93%. AMT kinetic parameters were also able to distinguish lung and breast cancer metastases, whereas MRI did not allow such distinction.

My last project involved assessment of prognostic value of AMT-PET in previously treated high-grade gliomas. AMT has previously proved capable of accurate differentiation of radiation necrosis and recurrent tumor, however it was unknown how this translates to survival information. Therefore, we studied various AMT-PET parameters in grade III-IV astrocytic brain tumors and correlated these with 1-year and overall survival (Juhász et al., 2013; Kamson et al., 2013a; Kamson et al., 2014). We found multiple AMT parameters that predicted both 1-year and overall survival. Patients with low AMT-uptake were more than 20-times more likely to be alive one year after the PET, and had a 5-times longer overall survival than those with high uptake.
The prognostic information provided by AMT-PET was independent of age and tumor grade (III vs. IV). Furthermore, AMT parameters could provide highly prognostic information in a group of patients whose disease was in a progressive phase as indicated by MRI.

In summary, AMT-PET appears to be a very promising tool for surgical and radiation planning as well as for follow-up of brain tumors. Despite the indirect evidence, the involvement of IDO and the kynurenine pathway in the increase of AMT uptake is still not clear. Nevertheless, AMT poses as a potential imaging marker for identification of patients who would benefit from the administration of novel drugs targeting IDO and/or the kynurenine pathway.
PAPER II.

Tryptophan PET in pretreatment delineation of newly-diagnosed gliomas: MRI and histopathologic correlates.


*Journal of Neurooncology*
(Kamson et al., 2013b)

PROBLEM

- Gliomas, especially high-grade, are infiltrative tumors
- Totality of initial glioma resection is an important factor in glioma survival
- On conventional MRI, T1-gad under-, T2/FLAIR overestimates tumor volume

GOAL

- To find an imaging modality that is capable of accurate assessment of tumor volume and infiltration

APPROACH

- AMT-PET, T1-gad, and T2/FLAIR defined tumor volumes were assessed in grade II-IV gliomas
- Spatial concordance and discordance was assessed between the modalities
- Tumor presence was histologically verified in concordant and discordant regions

FINDINGS

- AMT-defined volumes were larger than the T1-gad but smaller than the T2/FLAIR volume
- AMT volume was discordant with MRI increased with tumor proliferative index
- All samples contained tumor cells within but not outside the AMT-defined volume
- Tumor cell concentration was highest within the concordant AMT-T1-gad volume
Tryptophan PET in pretreatment delineation of newly-diagnosed gliomas: MRI and histopathologic correlates

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Abstract Pretreatment delineation of infiltrating glioma volume remains suboptimal with current neuroimaging techniques. Gadolinium-enhanced T1-weighted (T1-Gad) MR images often underestimate the true extent of the tumor, while T2-weighted images preferentially highlight peritumoral edema. Accumulation of $\alpha$-[11C]methyl-L-tryptophan (AMT) on positron emission tomography (PET) has been shown in gliomas. To determine whether increased uptake on AMT–PET would detect tumor-infiltrated brain tissue outside the contrast-enhancing region and differentiate it from peritumoral vasogenic edema, volumes and spatial concordance of T1-Gad and T2 MRI abnormalities as well as AMT–PET abnormalities were analyzed in 28 patients with newly-diagnosed WHO grade II-IV gliomas. AMT-accumulating grade I meningiomas were used to define an AMT uptake cutoff threshold that detects the tumor but excludes peri-meningioma vasogenic edema. Tumor infiltration in AMT-accumulating areas was studied in stereotactically-resected specimens from patients with glioblastoma. In the 28 gliomas, mean AMT–PET-defined tumor volumes were greater than the contrast-enhancing volume, but smaller than T2 abnormalities. Volume of AMT-accumulating tissue outside MRI abnormalities increased with higher tumor proliferative index and was the largest in glioblastomas. Tumor infiltration was confirmed by histopathology from AMT-positive regions outside contrast-enhancing glioblastoma mass, while no or minimal tumor cells were found in AMT-negative specimens. These results demonstrate that increased AMT accumulation on PET detects glioma-infiltrated brain tissue extending beyond the contrast-enhanced tumor mass. While tryptophan uptake is low in peritumoral vasogenic edema, AMT–PET can detect tumor-infiltrated...
brain outside T2-lesions. Thus, AMT–PET may assist pre-treatment delineation of tumor infiltration, particularly in high-grade gliomas.

**Keywords** Glioma · MRI · Positron emission tomography · Tryptophan · Volumetry · Vasogenic edema

**Introduction**

Prognosis of malignant gliomas remains bleak, and low-grade gliomas also pose a major diagnostic and therapeutic challenge [1, 2]. The high recurrence rate in infiltrating gliomas is in part caused by incomplete resection and irradiation of the tumor [3]. The vast majority (>80%) of tumor recurrence occurs within 2-cm of the resection margin [4, 5]. Extent of resection is a major predictor of outcome [6], however, assessing how much of the tumor is amenable to resection remains suboptimal. Currently, morphological MRI sequences such as post-gadolinium T1-weighted (T1-Gad), T2-weighted, and fluid-attenuated inversion recovery (FLAIR) imaging remain the mainstay of diagnosis, treatment planning, and follow-up for patients with gliomas [7, 8]. Gadolinium enhancement is a sensitive indicator of blood–brain barrier disruption [8, 9], but gliomas can infiltrate adjacent brain tissue without blood–brain barrier breakdown [7]. T2-weighted and FLAIR sequences reveal peritumoral edema. However, it is often difficult to differentiate a hyperintense T2/FLAIR signal related to a non-enhancing tumor from other etiologies (e.g., vasogenic edema or ischemic injury) [10].

Positron emission tomography (PET) provides signals based on the underlying biological activity. Amino acid PET may be a useful tool for microsurgical and radiotherapy planning in patients with high-grade gliomas [11–16]. α-[18F]methyl-l-tryptophan (AMT) is an amino acid PET tracer not incorporated into proteins; rather, it is metabolized via the immunomodulatory kynurenine pathway, involved in the escape of tumors from the host immune response [17–24]. In our previous studies, we noted increased AMT uptake in the majority of low-grade gliomas and in all high-grade gliomas [21, 25, 26].

In the present study, we assessed if AMT accumulation on PET could identify tumor-infiltrated brain tissue not detected by conventional MRI in newly-diagnosed gliomas. We hypothesized that these tumors would demonstrate increased AMT accumulation beyond the gadolinium-enhancing region and, in some cases, beyond T2 abnormalities in tumor-infiltrated brain tissue, while areas of vasogenic edema would not show elevated AMT uptake. We also hypothesized that the volume of AMT accumulation outside the MRI-defined abnormality would increase with higher histologic grade and tumor proliferative activity. Histopathology from stereotactically-resected brain tissue was performed to determine if AMT accumulation indeed detects tumor-infiltrated brain tissue in high-grade gliomas.

**Methods**

**Subjects**

We studied 28 adults with a newly-diagnosed glioma (Table 1) based on the following inclusion criteria: (1) a solid supratentorial mass on MRI; (2) no prior treatment; (3) subsequent microsurgical resection with WHO grade II–IV glioma. Twenty patients presented with seizures. The mean interval between AMT–PET and MRI was 19 days for low-grade and 4 days for high-grade gliomas. In addition, newly-diagnosed grade I meningiomas of 4 adult patients (mean age 67 years) were used to establish an AMT uptake threshold to differentiate between solid tumor mass and peri-meningioma vasogenic edema. The study was approved by the Institutional Review Board of Wayne State University, and written informed consent was obtained from all participants.

**AMT–PET scanning protocol**

PET studies were performed using a Siemens EXACT/HR whole-body positron emission tomograph (Siemens Medical Systems, Knoxville, Tennessee). The AMT tracer was synthesized by using a high-yield procedure as outlined before [27]. The procedure for AMT–PET scanning has been described previously [21, 26, 28]. In brief, after 6 h of fasting, AMT (37 MBq/kg) was injected intravenously. At 25 min after tracer injection, a dynamic emission scan of the brain (7 × 5 min) was acquired. Measured attenuation correction, scatter, and decay correction was applied to all PET images. For visualization of AMT uptake, averaged activity images 30–55 min post-injection were created and converted to an AMT standardized uptake value (SUV) image. The PET image in-plane resolution was 7.5 ± 0.4 mm at full-width half-maximum (FWHM) and 7.0 ± 0.5 mm FWHM in the axial direction.

**MRI protocol**

Diagnostic MRI scans acquired nearest in time to the AMT–PET scan were used in this study. MRI was performed on a Siemens MAGNETOM Trio TIM 3.0 Tesla scanner (Siemens Medical Solutions, Malvern, Pennsylvania) in 20 patients and on a GE Signa HDxt 3.0 Tesla scanner (GE Medical Systems, Milwaukee, Wisconsin) in eight. The following axial sequences (with similar parameters on both
scanners) were used for analysis: T2-weighted, FLAIR, and post-contrast T1-weighted (T1-Gad) images. FLAIR images were not acquired close to the time of AMT–PET in three patients. In patients with both T2 and FLAIR images acquired close to the PET scan, T2- and FLAIR-defined tumor volumes (see below) showed a very strong positive correlation ($r = 0.95; p < 0.001$); therefore, we used T1-Gad and T2 MR (but not FLAIR) for further image analysis.

### Image analysis

The 3D Slicer software version 3.6.3 ([www.slicer.org](http://www.slicer.org)) was used for threshold-based volume of interest (VOI) analysis [29]. First, AMT–PET and T2 images were co-registered to the T1 image volumes using the Fast Rigid Registration module [30]. Fused images were automatically resliced and resampled. Subsequently, we defined the threshold for

### Table 1: Clinical data and imaging abnormalities of the 28 glioma and 4 meningioma patients

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age/sex</th>
<th>Seizures</th>
<th>Tumor grade</th>
<th>Tumor histology</th>
<th>Tumor location</th>
<th>SUV-ratio (tumor/cortex)</th>
<th>SUV$_{\text{max-ratio}}$ (tumor/cortex)</th>
<th>Gad. Enh.</th>
<th>Volume of imaging abnormality (cm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>57/M</td>
<td>Yes</td>
<td>II</td>
<td>Astro</td>
<td>T</td>
<td>1.54</td>
<td>1.94</td>
<td>No</td>
<td>25.5</td>
</tr>
<tr>
<td>2</td>
<td>20/M</td>
<td>Yes</td>
<td>II</td>
<td>Oligo</td>
<td>F</td>
<td>~</td>
<td>1.18</td>
<td>No</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>32/F</td>
<td>No</td>
<td>II</td>
<td>Oligo</td>
<td>F</td>
<td>~</td>
<td>1.18</td>
<td>No</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>37/F</td>
<td>Yes</td>
<td>II</td>
<td>Oligo</td>
<td>P</td>
<td>1.65</td>
<td>2.48</td>
<td>No</td>
<td>5.7</td>
</tr>
<tr>
<td>5</td>
<td>34/M</td>
<td>Yes</td>
<td>II</td>
<td>Oligo</td>
<td>F</td>
<td>1.63</td>
<td>2.73</td>
<td>No</td>
<td>14.2</td>
</tr>
<tr>
<td>6</td>
<td>30/M</td>
<td>No</td>
<td>II</td>
<td>Oligo</td>
<td>F</td>
<td>1.76</td>
<td>2.53</td>
<td>No</td>
<td>63.2</td>
</tr>
<tr>
<td>7</td>
<td>50/M</td>
<td>Yes</td>
<td>III</td>
<td>Astro</td>
<td>P</td>
<td>~</td>
<td>1.17</td>
<td>No</td>
<td>0.0</td>
</tr>
<tr>
<td>8</td>
<td>36/M</td>
<td>Yes</td>
<td>III</td>
<td>Astro</td>
<td>F</td>
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<td>2.49</td>
<td>No</td>
<td>10.5</td>
</tr>
<tr>
<td>9</td>
<td>26/F</td>
<td>No</td>
<td>III</td>
<td>Oligo</td>
<td>F</td>
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<td>1.89</td>
<td>No</td>
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~ did not reach the pre-defined threshold; Astro astrocytoma, Oligo oligodendroglioma, GBM glioblastoma, F frontal, P parietal, T temporal, O occipital, Gad. Enh. gadolinium contrast enhancement, V$_{\text{AMT}}$, V$_{T2}$, V$_{T1-Gad}$ volumes of the AMT–PET, T2 and gadolinium enhancing abnormalities, respectively.
detecting increased AMT uptake, based on AMT SUV increases derived from the four WHO grade I meningiomas, surrounded by peritumoral vasogenic edema with no or minimal tumor cell infiltrate [31]. These meningiomas showed high AMT accumulation with an AMT SUV between 3.5 and 4.1, and tumor/contralateral cortex SUV ratios between 1.51 and 1.63; similar to SUV ratios in the gliomas (1.65 ± 0.26). Using co-registered, fused AMT–PET and MR images of the meningiomas, we determined that with an AMT cutoff of 36% above mean cortical SUV, AMT–PET and gadolinium-defined tumor volumes overlapped completely (i.e., peri-meningioma edema on T2/FLAIR was not detected) (Fig. 1a). Therefore, we used this cutoff threshold to delineate areas with increased AMT uptake in glioma patients, where regions with increased AMT SUV (>36% increase) were defined, and their volumes were expressed in cm³. In addition, the average AMT SUV and the maximal AMT SUV (SUV_max) within these PET volumes was calculated and compared to SUV in contralateral cortex. The site of highest AMT SUV was also determined.

Tumor volumes on MRI were defined as follows: (i) thresholds slightly above the highest signal of normal white matter contralateral to the tumor were used for T2 images; and (ii) area of contrast enhancement was used for T1-Gad images. VOIs (V-T1-Gad, V-T2) were created by segmentation of T1-Gad abnormalities semi-automatically, while T2 images were segmented manually to avoid erroneous inclusion of cerebrospinal fluid in the VOI.

Spatial concordance among imaging abnormalities was determined by calculating the number of overlapping abnormal voxels on both AMT and MRI (T2, T1-Gad) volumes. This was achieved by masking the VOI of AMT increase with the VOIs of the T2 abnormality and T1-Gad enhancement separately using 3D Slicer’s Mask module. Spatial discordance was determined by subtracting the volumes of overlaps from the AMT and MRI abnormality volumes using the Subtract module of 3D Slicer.

Neurosurgical planning, resection, and histopathologic examination

In order to assess histopathologic correlates of MRI and AMT–PET abnormalities, AMT–PET and MR images were used for neurosurgical planning and intraoperative navigation on a Brainlab Curve™ Image Guided Surgery platform (Brainlab Inc, Westchester, Illinois) in the five most recent patients with glioblastoma (#20, #23–26; Table 1). Due to the retrospective nature of this study, stereotactically obtained AMT–PET correlated samples were not available from the other patients. Using fused MRI/PET images from the five patients, samples were acquired stereotactically from regions with increased AMT SUV both with and without contrast enhancement (i.e., T1-Gad+/AMT+ and T1-Gad−/AMT− areas). In addition, T2-positive tissue samples outside the AMT-positive area were also obtained in two patients. Routine histopathologic analysis was performed and tumor infiltration was assessed from all stereotactically obtained specimens by an experienced neuropathologist (W.J.K.). The Ki-67 labeling index (%) was determined in the solid tumor tissue [32]. Presence and density of tumor cells was assessed in each stereotactically acquired specimen as follows: (i) solid tumor tissue (score 4); (ii) tumor-infiltrated brain tissue with high, intermediate, or low tumor cell density (scores 3, 2, and 1, respectively); and (iii) brain tissue with no/minimal tumor involvement (score 0).

Study design and statistical analysis

Most variables showed a non-normal distribution, therefore, non-parametric tests were used. The Wilcoxon signed-rank test was performed to compare tumor volumes derived from the different imaging modalities and also to compare tumor cell density scores in T1-Gad+/AMT+ versus T1-Gad−/AMT+ regions. AMT-accumulating MRI-negative tumor volumes were compared between glioma grades using the Mann–Whitney U test. Group correlations were done by Spearman’s rank correlation. Statistical analysis was carried out using the SPSS Statistics 19 software (SPSS Inc., Somers, New York). p values <0.05 were considered to represent statistical significance.

Results

Comparison of PET and MRI volume abnormalities

All 28 gliomas showed increased T2 signal; 24 gliomas also showed AMT accumulation with the 36% threshold of increased uptake. The four exceptions included one anaplastic
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astrocytoma (#7), one anaplastic oligodendroglioma (#11), and two low-grade oligodendrogliomas (#2 and #3) (Table 1). Gadolinium enhancement was noted in 14 patients (including 13 glioblastomas). Mean $V_{-T1-Gad}$ ($8.4 \pm 14.3$ cm$^3$) was much smaller than both $V_{-T2}$ ($39.7 \pm 33.6$ cm$^3$; $p = 0.001$) and $V_{-AMT}$ ($26.8 \pm 19.9$ cm$^3$; $p = 0.002$); $V_{-AMT}$ was smaller than $V_{-T2}$ ($p = 0.045$) (Fig. 2). In individual patients, the $V_{-T2}$ was greater than $V_{-AMT}$ in 20 patients, while the reverse was seen in the remaining eight (Table 1).

Spatial concordance between MRI and AMT–PET abnormalities

In gadolinium-enhancing tumors ($n = 14$), the enhancing tissue volumes on MRI were encompassed in the AMT-accumulating brain volumes, which were larger in all cases. Also, the site of highest AMT SUV was within or in very close vicinity to the gadolinium-enhancing tumor mass. In contrast, there was a variable spatial discordance between AMT-accumulating and T2-positive volumes.

In the 20 patients with WHO grade II–IV gliomas, the combined AMT/T2 abnormalities had an average volume of $54.6$ cm$^3$ ($\pm 32.4$ cm$^3$), with the bulk consisting of T2 abnormalities outside (mean: $23.3 \pm 27.2$ cm$^3$) or within (mean: $19.6 \pm 14.2$ cm$^3$) brain volumes showing high AMT uptake; approximately 23% of the combined mean volume showed pure AMT abnormalities (mean: $10.7 \pm 9.7$ cm$^3$) outside the T2 abnormalities (Fig. 3a). There were high individual variations regarding concordance and discordance between volumes of T2 and AMT–PET abnormalities (see Fig. 3b).

The AMT-accumulating tissue, which extended beyond the T2-volume, was significantly larger in glioblastomas (mean: $14.3 \pm 9.4$ cm$^3$) as compared to grade II or III gliomas (which were not different from each other: $3.5$ and $4.3$ cm$^3$, in average, respectively) ($p < 0.001$) (Fig. 1b). Also, higher tumor Ki-67 labeling index was associated with more extensive AMT uptake increases outside the T2-volume (Spearman’s rho = 0.64; $p < 0.001$). Tumor/cortex AMT SUV$_{max}$ ratios correlated with Ki-67 labeling index ($r = 0.50$; $p < 0.01$).

Histopathological correlates of imaging abnormalities

Thirteen stereotactically-resected specimens were obtained from five patients with newly-diagnosed glioblastoma. Tumor cell density scores were highest in T1-Gad+/AMT+ specimens (mean score 3.4, range: 3–4) and lower in T1-Gad−/AMT− specimens (mean score: 2.0, range: 1–3) ($p = 0.04$) (see example on Fig. 4). T1-Gad−/AMT− specimens (from brain tissue showing increased signal on T2 images), from two patients, showed very low tumor density scores (0 and 1).

Discussion

In this study, we found that AMT accumulation on PET extends beyond the contrast enhancing glioma mass. In addition, while the overall volume of brain tissue showing high AMT uptake was often smaller than corresponding regions with increased T2 signal, high AMT accumulation often extended beyond areas of T2 abnormality, particularly in glioblastomas. This discrepancy suggests that areas with AMT accumulation represent tumor-infiltrated brain tissue, some of which is not detected by contrast-enhancement or T2-weighted images. This is supported by the histopathologic analysis of stereotactically-acquired tissue, where we confirmed highest density of tumor cells in AMT-positive tissue samples taken from the area of gadolinium-enhancement and medium tumor cell density in AMT-positive non-enhancing tissue; no or minimal tumor cell density was seen outside the AMT-positive brain tissue. Altogether, these results suggest that increased AMT uptake may be useful to enhance accurate delineation of glioma-infiltrated brain tissue for neurosurgical planning and postoperative radiation therapy.

Thresholding PET abnormalities for tumor detection

Glioma cells infiltrate the brain via a gradient, hence gliomas lack definitive margins and are associated with
infiltrative edema [3]. Therefore, defining a cutoff threshold for glioma definition on imaging requires a decision: for pathology-based thresholds one has to define what density of cell infiltration is important (or feasible) to be detected, and there may be different thresholds for different cell densities. Conversely, low-grade meningiomas are non-infiltrative tumors with a clear interface between tumor and underlying cortex, and are associated with vasogenic edema. Therefore, meningiomas have been used as a model for pure vasogenic edema [33]. Since low-grade meningiomas avidly accumulate AMT (similar to high-grade gliomas), they are useful to generate a cutoff threshold, which can pick up adequate tumor volume while excluding non-infiltrative edema (including peri-glioma edema with low tumor cell infiltration) and non-tumoral brain tissue. It is likely that the selected meningioma-based cutoff threshold (36 %) excludes some glioma-infiltrated brain tissue with relatively low tumor cell density. We have recently reported a preliminary PET study with pathology comparison suggesting that various tumor cell densities may be outlined by various thresholds on AMT–PET [34]; however, this needs further confirmation in larger samples, and only 13 specimens from five patients were used in the present study after the exclusion of patients with recurrent gliomas.

With the threshold established for the current study, we found decreased AMT uptake in peri-meningioma edema, which is consistent with a recent PET study that noted decreased methionine uptake in vasogenic edema [33]. Although we did not have histopathologic evidence of complete absence of infiltrating tumor cells in peri-meningioma regions, peritumoral invasion is generally observed only in high-grade meningiomas [31], which were not included in this study.

MRI abnormalities and AMT uptake

Despite inherent limitations of contrast-enhanced MRI [7, 8, 35, 36], the Macdonald Criteria are still widely used for monitoring brain tumor progression [37] and rely on changes in contrast enhancement [7]. In the present study, high AMT accumulation on PET (>36 %, as compared to normal cortex) extended variably beyond the area of contrast uptake in gliomas into non-enhancing regions, frequently in an eccentric fashion. In contrast, grade I meningiomas, which showed high AMT SUVs (and downward sloping time activity curves) similar to high-grade gliomas, showed no AMT uptake beyond the contrast enhancing mass when using the same threshold of increased AMT uptake. High AMT uptake observed outside the contrast-enhancing
glioma mass in the present study is likely caused by tumor infiltration of brain tissue without a major disruption of the blood–brain barrier. This notion is supported by our limited histopathologic analysis, where non-enhancing AMT-accumulating tissue tumor volume showed massive tumor cell presence.

T2 (and also FLAIR) MRI sequences have been recently incorporated in tumor follow-up protocols [7, 38]. We found a partial discordance between T2 and AMT–PET abnormalities. First, the overall extent of T2 abnormalities was larger than the area of AMT accumulation in most cases. It is likely that T2 signal abnormalities in areas with low AMT uptake mostly represent brain tissue with vasogenic edema. This is supported both by our findings in peri-meningioma edema and also our histology data from AMT-negative (but T2-positive) brain tissue, where no or very low tumor cell density was observed. A second interesting finding was the variable extension of increased AMT uptake beyond the T2 abnormality, especially in patients with highly proliferative gliomas. Again, the most plausible explanation for this is the presence of tumor cells beyond the regions defined by T2 MRI, in tumor-infiltrated brain tissue without detectable edema. This needs to be confirmed by further histopathologic studies of AMT+/T2– brain tissue.

Fig. 4 a AMT–PET co-registered to the post-contrast T1 (T1-Gad) image of patient #20 with a left parietal glioblastoma. b Three-dimensional surface reconstruction of the same subject’s brain, visualizing AMT uptake on a rainbow scale where red represents the highest and dark purple represents the lowest standard uptake values (SUVs). Tissue specimens were obtained with stereotactic guidance from the non-enhancing high AMT SUV region (red arrows) as well as the contrast-enhancing high AMT SUV area (yellow arrows). c, d Hematoxylin and eosin staining of the specimens from the non-enhancing high AMT SUV (c) and the enhancing high AMT SUV region (d). The arrows indicate examples of infiltrating neoplastic cells. Original magnification at ×20.
Potential mechanisms of increased AMT uptake in tumor-infiltrated brain tissue

\(\alpha\)-[\(\text{\textsuperscript{13}}\text{C}\)]methyl-l-tryptophan accumulation is not affected by protein incorporation [18]. Using dynamic AMT–PET images, we recently demonstrated that high net tryptophan transport in gliomas is an excellent predictor of glioma proliferative activity [32]. Increased tryptophan transport may occur due to overexpression of the LAT1 amino acid transporter; high LAT1 expression was in fact reported to correlate with high methionine uptake on PET in newly-diagnosed gliomas [39]. Therefore, increased AMT uptake outside the contrast-enhancing tumor mass may detect proliferative tumor cells overexpressing LAT1. However, high AMT transport due to LAT1 may not fully explain high SUVs measured in the late uptake phase (25–60 min after tracer injection, i.e., the time frame of brain scans in our study). High AMT SUV in this late phase may also be related to retention of tryptophan or its metabolite(s), such as l-kyurenine, due to elevated tumoral IDO expression [21], which facilitates increased tryptophan metabolism via the immunosuppressive kynurenine pathway. High IDO is not strongly related to tumor grade, as it has been observed in low-grade gliomas and was not present in some glioblastomas [21]; rather, IDO overexpression may create an immunosuppressive microenvironment. There could be other explanations for peritumoral cortical AMT SUV increases. For example, increased focal cerebral AMT uptake can be associated with epileptogenic regions, mostly seen around epileptogenic tubers and focal cortical malformations, but also reported in some patients with cryptogenic epilepsy [40–43]. However, in non-tumoral epilepsy cases, the degree of AMT accumulation rarely exceeded 20 %, and >30 % increased uptake was exceptional. Reactive gliosis can also cause increased tracer uptake as demonstrated with other amino acid tracers [44]. Similarly, mild increases of AMT uptake were occasionally observed in epileptic foci where histopathology revealed reactive gliosis, but these increases were rarely higher than 10 %, and did not reach the 36 % asymmetry threshold [42, 43]. Therefore, tumor definition by using the 36 % AMT cutoff threshold in our study is unlikely to be confounded by epileptogenic regions or reactive gliosis.

Comparison of AMT–PET to other amino acid PET tracers

Several previous PET studies of various (non-AMT) amino acid PET radiotracers, most commonly labeled methionine (MET) and O-[\(\text{\textsuperscript{18}}\text{F}\)]fluoroethyl-l-tyrosine (FET), showed accumulation beyond areas of gadolinium enhancement [8, 12, 13, 16, 45, 46]. Such studies suggested that amino acid PET imaging may enhance target delineation for surgery and subsequent radiotherapy of gliomas [15, 45–47]. Identification of high-uptake foci in low-grade gliomas may also be useful to identify anaplastic foci. Four of 6 grade II gliomas had SUV\(_\text{max}\) in the range of mean SUV of high-grade tumors. Considering the positive correlation between AMT SUV and tumor proliferative index, these high SUV\(_\text{max}\) foci may represent anaplastic regions within a low-grade tumor. Anaplastic tumor foci have been demonstrated in low-grade gliomas based on differing FET–PET uptake kinetics in a previous study [48].

The degree of observed increases of PET tracer uptake and the applied threshold varies moderately across different studies and tracers. Most MET-PET studies used cutoff values between 1.27 and 1.5 above the uptake of the control region [8, 45, 49, 50]. Using histopathologic sampling, Kracht et al. [50] established a cutoff tumor-to-gray matter ratio of 1.3 for tumor delineation with MET, while Pauleit et al. [51] found a ratio of 1.6 to have 92 % sensitivity and 81 % specificity on FET–PET. Grosu et al. [45] showed great concordance of volumes between FET and MET using a 1.5 cutoff. It should be noted, however, that direct comparison of values across studies and tracers is difficult, because different groups use tumor-to-control ratios variably utilizing the mean or maximal SUV for the tumor, and defining white or gray matter or a composite of the two as the control region. We have used cortical SUV to generate the ratios; the ratios would be higher if white matter or a mixed white/gray matter tissue would be used. Although the 36 % threshold for increased AMT uptake defined in the present study is within the range of previous MET and FET cutoff values, the optimal cutoff threshold for increased uptake could be slightly different among various amino acid radiotracers, considering the different mechanism of uptake and metabolism. Nevertheless, our histopathologic studies, albeit not as extensive as the one presented by Pauleit et al. [51] for FET–PET, strongly suggest that delineation of brain regions with increased uptake of AMT can provide added clinical information to conventional MRI regarding tumor extent. In addition, considering the infiltrative nature of malignant gliomas, without a sharp tumor border, it is likely that different cutoff thresholds may delineate brain tissue with various tumor cell infiltration. Less than 36 % increases of AMT uptake could be informative in detecting brain regions with low tumor cell density and further increase the clinical yield of pretreatment AMT–PET. This will require further rigorous comparisons between radiotracer uptake and histopathology of stereotactic tissue samples. Nevertheless, as AMT–PET imaging detects regions of possible tumor infiltration not revealed by conventional MRI, its integration into treatment planning and/or follow-up imaging holds the promise of better tailored resections, more accurate radiotherapeutic targeting, and/or better assessment of tumor recurrence. Improvements in
any of these areas could enhance prognosis. Prior studies with amino acid PET tracers indeed provided preliminary data suggesting that combining PET imaging with MRI in tumor volume delineation may yield better outcomes than using conventional MRI alone [8, 12–16]. Tumoral transport of tryptophan, methionine, tyrosine, and other large neutral amino acids, whose derivatives are being used for PET imaging is likely determined by similar transport mechanisms (mediated by LAT1, see above). Currently, there is no evidence that any of the amino acid tracers are superior to the others for brain tumor detection. From a practical point of view, 18F-labeled tracers (such as FET) are more suited for distribution and clinical use. Centers with an on-site cyclotron might prefer a particular 11C-labeled tracer, e.g., if the tracer can be used for multiple purposes (such as AMT for detecting epileptic foci). Also, each of these PET radiotracers has different metabolic fates; therefore, there may be tumor types or special situations where one performs better than the others. Further quantification of uptake kinetics may provide additional information to refine the role of amino acid PET radiotracers in presurgical delineation and postsurgical monitoring of gliomas.

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Conflict of interest None of the authors report any conflict of interest or financial disclosure.

References


 Springer


PAPER III.

Differentiation of glioblastomas from metastatic brain tumors by tryptophan uptake and kinetic analysis: a positron emission tomographic study with magnetic resonance imaging comparison.

Kamson, D. O., Mittal, S., Buth, A., Muzik, O., Kupsky, W. J., Robinette, N. L., Barger, G. R., Juhász, C.

*Molecular Imaging*  
(Kamson et al., 2013c)

PROBLEM

- Solitary metastases and glioblastomas may appear very similar on conventional MRI
- In 15% of brain metastases, the primary tumor source is unknown
- Preoperative diagnosis may alter the course of treatment and therefore, the outcome

GOAL

- To differentiate metastatic brain tumors from glioblastomas
- To differentiate brain metastases based on origin

APPROACH

- Various AMT-PET kinetic parameters were quantified within brain tumors
- AMT parameters were tested for differentiation between brain tumors of different origin

FINDINGS

- Kinetic AMT parameters could accurately differentiate glioblastomas from metastases
- Combination of AMT-PET and T1-gad increased the differentiating accuracy to 93%
- AMT-PET could also distinguish lung vs. breast metastases, whereas T1-gad could not
Differentiation of Glioblastomas from Metastatic Brain Tumors by Tryptophan Uptake and Kinetic Analysis: A Positron Emission Tomographic Study with Magnetic Resonance Imaging Comparison

David O. Kamson, Sandeep Mittal, Amy Buth, Otto Muzik, William J. Kupsky, Natasha L. Robinette, Geoffrey R. Barger, and Csaba Juhašz

Abstract

Differentiating high-grade gliomas from solitary brain metastases is often difficult by conventional magnetic resonance imaging (MRI); molecular imaging may facilitate such discrimination. We tested the accuracy of \( ^{11}\text{C}\)-methyl-L-tryptophan (AMT)-positron emission tomography (PET) to differentiate newly diagnosed glioblastomas from brain metastases. AMT-PET was performed in 36 adults with suspected brain malignancy. Tumoral AMT accumulation was measured by standardized uptake values (SUVs). Tracer kinetic analysis was also performed to separate tumoral net tryptophan transport (by AMT volume of distribution [VD]) from unidirectional uptake rates using dynamic PET and blood input function. Differentiating the accuracy of these PET variables was evaluated and compared to conventional MRI. For glioblastoma/metastasis differentiation, tumoral AMT SUV showed the highest accuracy (74%) and the tumor/cortex VD ratio had the highest positive predictive value (82%). The combined accuracy of MRI (size of contrast-enhancing lesion) and AMT-PET reached up to 93%. For ring-enhancing lesions, tumor/cortex SUV ratios were higher in glioblastomas than in metastatic tumors and could differentiate these two tumor types with > 90% accuracy. These results demonstrate that evaluation of tryptophan accumulation by PET can enhance pretreatment differentiation of glioblastomas and metastatic brain tumors. This approach may be particularly useful in patients with a newly diagnosed solitary ring-enhancing mass.

IN ADULTS, metastatic tumors (eg, from primary lung cancer, breast cancer, and malignant melanoma) and glioblastoma multiforme (GBM) are the most common malignancies in the brain.\(^1,2\) Despite the poor outcomes of both primary and metastatic brain tumors,\(^1,3\) their distinction is important because of their substantially different clinical management. Although the standard treatment of GBM involves intracranial microsurgery with adjuvant radiation with concomitant chemotherapy,\(^4,5\) metastatic brain disease is commonly treated with upfront stereotactic radiosurgery and/or whole-brain radiation.\(^1\) Conventional magnetic resonance imaging (MRI) is highly sensitive for detection of these tumors, but the specificity of the MRI findings remains limited. All of these tumors can show enhancement on \( T_1 \) -weighted images with gadolinium (\( T_1 \)-Gad) and hyperintensity on \( T_2 \) and fluid-attenuated inversion recovery (FLAIR) images.\(^6,7\) The limited specificity of MRI for the differentiation of GBMs from brain metastases poses a particularly difficult clinical dilemma when a single brain lesion, suspicious for a high-grade neoplasm, is found in a patient without a history of a known primary tumor.\(^6\) There are general characteristic imaging patterns of brain metastases, such as the invasion of the cortical gray-white matter junction and the presence of multiple nodular enhancing lesions,\(^6,8\) whereas GBM most commonly presents as a larger solitary ring-enhancing lesion within the deep white matter. However, differentiation of metastases from high-grade gliomas can be problematic if the primary tumor is unknown, which occurs in up to 15% of patients with
suspected brain metastasis. Additionally, large solitary metastases within the deep brain with ring enhancement are not uncommon.

Positron emission tomography (PET) may offer useful complementary information in diagnosis and differentiation of some malignant brain tumors because PET provides signals based on tissue metabolic activity, which may differ among various tumor grades and types. Clinical PET with 2-deoxy-2[18F]fluoro-d-glucose (FDG) is often used to differentiate low-grade from high-grade brain tumors but provides limited accuracy in differentiating among the various high-grade tumor types, which show high glucose uptake. Amino acid PET tracers have been shown to be useful in glioma treatment planning and follow-up. A limited amount of data is also available on uses of such tracers in metastatic brain disease. However, we are not aware of any PET studies successfully attempting to differentiate glial and metastatic brain tumors.

In the present study, we used α-[11C]-methyl-L-tryptophan (AMT)-PET to study primary and metastatic brain tumors. AMT is an amino acid PET tracer not incorporated into proteins; instead, tryptophan (and also AMT) is transported in brain tumor tissue via the large neutral amino acid transporter (LAT1) and can be metabolized via the immunomodulatory kynurenine pathway, which plays a key role in tumoral escape from the host immune response. Recent studies also suggest that this pathway plays a prominent role in brain tumor pathogenesis. In our previous studies, we have shown that AMT tracer uptake is increased in most low-grade gliomas and in all high-grade gliomas, and kinetic analysis of AMT transport and metabolism on dynamic PET images was able to differentiate among various glioma types and predict proliferative activity. AMT tracer kinetic analysis can also facilitate accurate differentiation between glioma recurrence and radiation injury after initial treatment. In addition, we have found differential AMT kinetics on PET imaging in primary lung and breast cancers (two common cancer types that often metastasize to the brain), likely owing to their differences in tumoral blood flow, tryptophan transporter activity, and mode of intratumoral tryptophan metabolism (eg, via the kynurenine and/or serotonin pathways), as well as other, yet to be clarified mechanisms. Therefore, in the present study, we hypothesized that quantitative analysis of AMT uptake and kinetics may be able to differentiate GBMs from common metastatic brain tumors and, thus, supplement conventional diagnostic neuroimaging. Such differentiation may be of significant clinical value in selected patients to guide further diagnostic and therapeutic steps.

### Methods

#### Subjects

We studied 43 brain tumors in 36 adults (Table 1) based on the following inclusion criteria: (1) at least one lesion suggesting a brain tumor on MRI; (2) for GBMs, no previous treatment; for metastases, no previous treatment targeting the current (new) metastatic brain lesion and its vicinity and/or no whole-brain radiation performed in at least 12 months prior to the PET imaging; and (3) subsequent craniotomy with microsurgical resection and the histopathologic diagnosis of GBM or metastasis or histopathologic evidence from the site of the primary extracranial tumor (patients 20, 27, and 36). The mean time interval between the AMT-PET and MRI was 5 days (range 0–13 days) for the metastasis group and 4 days (range 0–17 days) for the GBM group. The study was approved by the Institutional Review Board of Wayne State University, and written informed consent was obtained from all participants.

#### AMT-PET Scanning Protocol

PET studies were performed using a Siemens EXACT/HR whole-body positron emission tomograph (Siemens Medical Systems, Knoxville, TN). The PET image in-plane resolution was 7.5 ± 0.4 mm at full-width half-maximum (FWHM) and 7.0 ± 0.5 mm FWHM in the axial direction. The AMT tracer was synthesized by using a high-yield procedure as outlined previously. The procedure for AMT-PET scanning has been described previously. In brief, after 6 hours of fasting, a slow bolus of AMT (37 MBq/kg) was injected over 2 minutes via a venous line. A second venous line was established for collection of timed blood samples (0.5 mL/sample, collected at 20, 30, 40, 50, and 60 minutes after AMT injection). Initially, coinciding with tracer injection, a 20-minute dynamic PET scan of the heart was performed to obtain the blood input function from the left cardiac ventricle. The blood input function was continued beyond this initial 20 minutes by using venous blood samples as described previously. At 25 minutes after tracer injection, a dynamic emission scan of the brain (7 × 5 minutes) was acquired. Measured attenuation correction, scatter, and decay correction were applied to all PET images. For visualization of AMT uptake, averaged activity images 30 to 55 minutes postinjection were created and converted to an AMT standardized uptake value (SUV) image. For quantification of the AMT net transport and metabolism, Patlak graphical analysis was performed, which yielded AMT volume of distribution (VD) and AMT K values, as...
described previously.\textsuperscript{29,32} VD is an estimate of the volume of distribution of the tracer in the free precursor pool. We have previously shown that tumoral VD is increased when the blood-brain barrier is compromised,\textsuperscript{29} and tumoral VD was also a good estimate of tumor proliferative activity in newly diagnosed gliomas.\textsuperscript{31} The AMT $K$ value reflects the

<table>
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<th>Patient No.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Tumor Location</th>
<th>Tumor Primary</th>
<th>Pattern of Enhancement</th>
<th>Tumoral Value</th>
<th>Tumor/Cortex Ratio</th>
<th>$T_{1}$-Gad Diameter (mm)</th>
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</tbody>
</table>

C = cerebellar; F = frontal; MRI = magnetic resonance imaging; O = occipital; P = parietal; SUV = standardized uptake value; T = temporal; VD = volume of distribution.
unidirectional uptake of tracer into tissue, which is thought to be proportional to the metabolism of tryptophan via the serotonin (in normal brain) and/or the kynurenine pathway.

### MRI Protocol

Pretreatment diagnostic MRI scans acquired nearest in time to the AMT-PET scan were used in this study. MRI was performed on either a Siemens MAGNETOM Trio TIM 3.0 Tesla scanner (Siemens Medical Solutions, Malvern, PA), a GE Signa HDxt 3.0 Tesla scanner (GE Medical Systems, Milwaukee, WI), or a Philips Achieva TX 3.0 Tesla scanner (Philips Medical Systems Inc., Da Best, the Netherlands). Postcontrast T1-weighted images used in this study were acquired using similar parameters on all scanners. The pattern of gadolinium enhancement was categorized as (1) ring enhancement; (2) nodular enhancement; (3) other pattern of enhancement if it did not fit into either of the above categories; or (4) no enhancement.

### Image Analysis

The 3D Slicer software version 3.6.3 (http://www.slicer.org) was used for threshold-based region of interest (ROI) analysis. First, a transformation matrix was created by coregistration of the summed AMT-PET images to the T1-Gad image volumes using the Fast Rigid Registration module. This transformation matrix was then applied to the summed AMT-PET image and to the dynamic AMT-PET images loaded via the 4D Image module of 3D Slicer. Following fusion of the summed AMT-PET with T1-Gad images, all lesions showing a clear increase in AMT uptake in locations corresponding to abnormal gadolinium enhancement were identified visually. The largest enhancing diameter on T1-Gad was measured. Lesions smaller than 3.5 mm on MRI (present in patient 33 with multiple melanoma metastases) did not show increased AMT uptake and were not analyzed further. Lesions larger than 7 mm (ie, a size consistent with the FWHM for AMT-PET scans) in the largest diameter were quantitatively analyzed using 3D Slicer’s edit module in the following manner: tumoral ROIs were drawn on the AMT-PET/MRI fusion image (axial planes) on regions showing abnormal gadolinium enhancement and/or abnormal AMT increase and on the contralateral homotopic cortical region. If AMT increase extended beyond a T1-Gad-positive lesion, the entire region showing increased AMT uptake was included in the ROI. If multiple tumors were present, the contralateral reference cortical region for the largest lesion was used as the reference region for the smaller cerebral lesions as well, whereas cerebellar lesions had a reference region obtained from the homotopic cortex of the contralateral cerebellar hemisphere. T1-Gad negative lesions were identified on AMT-PET images by having an SUV higher than the reference cortical region and were further confirmed by newly developed abnormalities in these locations on subsequent follow-up MRI. Subsequently, the mean AMT SUV and kinetic parameters were quantified from each ROI, and tumoral SUV, K, and VD values, as well as SUV, K, and VD tumor/cortex ratios, were calculated for each lesion as the average of up to three ROI (depending on lesion size) with the highest mean SUV.

### Study Design and Statistical Analysis

Most variables showed a nonnormal distribution; therefore, nonparametric tests were used. The Mann-Whitney U test was used for group comparison (GBMs vs metastases; GBMs vs carcinomas; GBMs vs lung metastases; GBMs vs breast metastases; lung vs breast metastases; ring-enhancing metastases vs ring-enhancing GBMs; ring-enhancing vs nodular-enhancing metastases). The following PET parameters of the lesions were compared: tumor SUV, K, and VD, as well as tumor/cortex SUV, K, and VD ratios. In case of a significant difference in any of these parameters between the tumor groups, receiver operating characteristic (ROC) analyses were performed to determine the optimal threshold for the distinction of tumor types. Accuracy and positive predictive values (PPVs) were calculated by using these thresholds. In the comparison of GBMs and metastases, concurrent diagnoses suggested by AMT-PET versus clinical diagnoses were classified as follows: true positive (TP) = AMT and clinical diagnosis of GBM; true negative (TN) = AMT and clinical diagnosis of metastasis; false positive (FP) = AMT diagnosis of GBM with clinical diagnosis of metastasis; false negative (FN) = AMT diagnosis of metastasis with clinical diagnosis of GBM; and PPV referring to the correct prediction of GBM. In the comparison of lung versus breast metastases, concurrent AMT and clinical diagnoses of lung metastasis were considered TP, and so on; in this comparison, PPV referred to the prediction of lung metastasis. Accuracy was calculated as TP/(TN + TP + FN + FP), whereas PPV was determined as TP/(TP + FP); both were indicated as percentages. Statistical analysis was carried out using SPSS Statistics 19 software (SPSS Inc., Somers, NY); p values < .05 were considered statistically significant.
Results

Imaging Characteristics of the Brain Tumors

On the scans of the 17 patients with metastases, 31 lesions were identified by T$_1$-Gad and/or AMT-PET. Gadolinium enhancement was seen in 30 lesions, and increased AMT uptake was detected in 28 lesions by visual assessment; all 28 showed at least a 16% SUV increase, compared to contralateral normal cortical uptake (range 1.16–3.21). All metastatic lesions $\geq 7$ mm on T$_1$-Gad ($n = 24$) showed AMT accumulation. One patient (24) had one AMT-negative lesion, which was 4 mm in diameter on T$_1$-Gad, and another patient (33) had multiple AMT-negative metastases measuring 2 to 3 mm in diameter on MRI. Thirteen metastatic lesions showed nodular enhancement, eight were ring enhancing, and two showed an enhancement pattern that did not fit into these two categories. The only nonenhancing metastatic lesion (a melanoma metastasis, patient 33d in Table 1) had high AMT SUV (tumor/cortex ratio 1.30), showed signal intensity very similar to that of the surrounding white matter on T$_1$-Gad, and only minimally distorted the gyral pattern. Follow-up MRI of this lesion 4 weeks later showed growth, suggesting tumor presence, although it still presented no clear contrast enhancement.

All 19 GBMs showed gadolinium enhancement and increased AMT uptake, with an SUV tumor/cortex ratio range of 1.37 to 3.33. Fourteen GBMs were ring enhancing, and five showed nodular enhancement.

Comparison of Different Tumor Types

<table>
<thead>
<tr>
<th>GBMs versus All Metastases</th>
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</thead>
</table>

Compared to GBMs, metastases had lower tumoral SUVs (mean ± SD: 3.84 ± 1.47 vs 5.02 ± 1.44; $p = .005$), as well as a lower mean tumor/cortex SUV ratio (mean ± SD: 1.78 ± 0.57 vs 2.10 ± 0.46; $p = .009$) and tumor/cortex VD ratio (mean ± SD: 2.07 ± 0.67 vs 3.09 ± 1.45; $p = .014$) (Table 2). For the distinction of GBMs and metastatic tumors, a tumoral SUV of 4.1 had the highest differentiating accuracy (74%) and a VD tumor/cortex ratio of 2.8 had the highest PPV (82%; Table 3). Tumor diameter on T$_1$-Gad MRI was greater in the GBM group compared to the metastases (40 ± 17 mm vs 25 ± 14 mm; $p = .003$). A diameter threshold of 28 mm separated the two tumor groups with 72% accuracy, but PPV was lower

Table 2. Comparisons of AMT Kinetic Parameters of Different Tumor Groups

<table>
<thead>
<tr>
<th>Tumoral SUV</th>
<th>GBMs</th>
<th>All Metastases</th>
<th>Carcinomas</th>
<th>Breast Metastases</th>
<th>Lung Metastases</th>
<th>Ring-Enhancing GBMs</th>
<th>Ring-Enhancing Metastases</th>
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AMT = [11C]methyl-L-tryptophan; GBM = glioblastoma; NS = not significant; SUV = standardized uptake value; VD = volume of distribution.

*p value of ring-enhancing GBM and ring-enhancing metastasis comparison.
than PPV obtained by AMT-PET. In cases where there was consensus in tumor classification by AMT SUV and the diameter on T<sub>1</sub>-Gad (based on the above-described cutoff threshold values, n = 26), the combined accuracy has increased to 88% with a PPV of 75%. The accuracy of combined MRI + AMT-PET classification reached 93% after the addition of nonconsensus cases with a high VD ratio (> 3.5) classified as GBMs.

**Differentiation of Ring-Enhancing GBMs and Metastases**

Ring-enhancing metastases showed a lower diameter on T<sub>1</sub>-Gad than ring-enhancing GBMs (27 ± 16 mm vs 43 ± 15 mm, respectively; p = .029). The tumor SUV and the tumor/cortex SUV, K, and VD ratios were all lower in the ring-enhancing metastases compared to GBMs (p values: .029, .001, .048, and .034, respectively; see Table 2). The SUV ratio of 1.65 reached the highest differentiating accuracy of 91%, with a PPV of 93% (see Table 3), falsely identifying only one metastasis as GBM (1 of 8) and one GBM as metastasis (1 of 14), whereas the diameter threshold of 27 mm produced a lower accuracy (82%) and PPV (81%).

**GBMs versus Metastatic Carcinomas**

After the exclusion of melanomas, the remaining metastatic carcinomas (n = 19) were compared to the GBMs. Tumoral SUV and K, VD, and SUV tumor/cortex ratios were all higher in GBMs (see Table 2). Tumoral SUV with a 4.1 threshold was the most accurate for the distinction of the two groups with an accuracy of 74% (PPV = 74%; see Table 3), and the highest PPV (90%) was provided by a VD tumor/cortex ratio of 2.8. The mean diameter was higher in the GBM group than in the carcinomatous metastases (40 ± 17 mm vs 25 ± 15 mm; p = .006). The diameter threshold of 28 mm separated the GBMs and carcinomas with 71% accuracy and 70% PPV.

**GBMs versus Breast Metastases**

Only VD tumor/cortex ratios differed between GBMs and breast metastases, with a higher mean value in GBMs (see Table 2). A VD ratio of 1.9 was able to distinguish the two groups with 84% accuracy and 94% PPV (see Table 3). Also, diameter difference was found on T<sub>1</sub>-Gad MRIs between GBMs and breast metastases (40 ± 17 mm vs 29 ± 17 mm; p = .026). The diameter threshold of 24 mm produced 77% accuracy and 84% PPV.

**GBMs versus Lung Metastases**

Lung metastases had significantly lower tumoral SUVs and K tumor/cortex ratios compared to GBMs (see Table 2). An SUV threshold of 4.1 distinguished the two groups,
with an 81% accuracy and 100% PPV, and a 1.52 tumor/cortex K ratio also had high accuracy (78%) and PPV (88%; see Table 3). The mean tumor diameter on $T_1$-Gad was higher in the GBM group than in the lung metastases (40 ± 17 mm vs 25 ± 15 mm; $p = .026$). A 28 mm diameter threshold separated the two groups with 74% accuracy and 88% PPV.

**Breast versus Lung Metastases**

The tumor/cortex VD ratios in the lung metastases were higher than in the breast metastases (2.35 ± 0.47 vs 1.78 ± 0.53, respectively; $p = .039$). A VD ratio threshold of 1.9 distinguished the two groups with 86% accuracy and 88% PPV. No diameter difference was found on $T_1$-Gad between lung and breast metastases (37 ± 20 mm vs 25 ± 15 mm, respectively; $p = .64$).

**Discussion**

In this study, we found that tumoral AMT uptake can distinguish GBMs from metastatic tumors with a high degree of accuracy. SUVs are easy to obtain without blood input function, and tumor/cortex AMT SUV ratios appear to be very accurate (> 90%) in discriminating ring-enhancing GBMs from brain metastases, a difficult clinical dilemma when a single new ring-enhancing lesion is demonstrated on MRI (Figure 1). Tumoral SUV could also distinguish GBMs from lung metastases with a very high positive predictive value. Kinetic analysis of AMT uptake provided useful additional information, compared to contrast-enhanced MRI ($T_1$-Gad diameter), for identifying GBMs based on their higher tumor/cortex AMT VD ratios. With the combination of a simple MRI parameter (size of contrast-enhancing lesion) and AMT-PET values, the differentiating accuracy reached up to 93%. These results suggest that AMT SUV and VD, measured by PET, can provide an improved discrimination of the most common malignant tumor types within the brain.

**Imaging Features to Differentiate Malignant Brain Tumor Types**

There are a few features on conventional MRI that can help differentiate primary gliomas from metastatic brain tumors. For example, localization in the gray-white matter junction is generally associated with metastases. In our study, this was not helpful in the distinction of tumor types because the majority of both GBMs and metastases penetrated the white matter and involved the cortex at the same time. Another typical feature of glial tumors is their larger diameter compared to metastases. The maximum diameter of enhancement was indeed greater in GBMs than in metastases in our study; however, no significant difference was found among different types of metastatic lesions. In addition, abnormalities on advanced MRI, including magnetic resonance spectroscopy (MRS) and perfusion-weighted imaging (PWI), may be helpful to differentiate infiltrative gliomas from other solid brain tumor types, which have different metabolite and blood flow characteristics in peritumoral edema. However, high-grade glial neoplasms and metastases often demonstrate increased relative cerebral blood volume on PWI. MRS has limited brain coverage even when using a multivoxel technique and is often nondiagnostic if the area imaged contains blood products or is too close to...
cerebrospinal fluid spaces or bone. Lastly, both MRS and PWI have poor spatial resolution. Therefore, these techniques currently have limited use in treatment planning, although they can improve posttreatment differentiation of recurrent gliomas from radiation injury. In contrast, PET scanning with various amino acid radiotracers is an excellent tool for detecting infiltrative tumor growth in high-grade glial tumors and can be directly used in stereotactic surgical and radiosurgical planning.

Compared to MRI, PET is less available and requires more expensive instrumentation, and the availability of \( ^{11} \)C-labeled amino acid radiotracers is limited to specialized centers equipped with a cyclotron. FDG-PET is more widely used but has a limited accuracy in high-grade brain tumor imaging. A thorough analysis of brain tumor cases from the National Oncologic PET Registry showed that dedicated FDG-PET scanning of the brain rarely changed the management; however, patients who underwent brain FDG-PET were biopsied much less often; thus, its economic efficacy might lie in FDG being a plausible surrogate for brain biopsy. In the present study, we found that AMT-PET can accurately distinguish most common high-grade tumors that invade brain parenchyma. It is generally thought that brain metastases are recognized earlier because they cause neurologic symptoms at a smaller size due to their vicinity to the cortex, and clinicians have a lower threshold of suspicion in patients whose primary tumor is already known. It is plausible that, in addition to the characteristic presence in the gray-white matter junction, the presence of nodular enhancement is an early morphologic feature of many metastatic tumors, and as tumoral development progresses (with angiogenesis at the tumor edge and central necrosis), the tumors adapt a ring-enhancing pattern, a morphology that converges toward the features of GBMs on conventional MRI. AMT-PET was found to have excellent accuracy for discrimination of tumors with such morphology; thus, it can be valuable in these challenging cases.

**Differentiation of AMT Kinetics of Lung versus Breast Metastases and Potential Mechanism of AMT Uptake Differences**

The present study provides preliminary evidence that quantification of AMT uptake may assist differentiation of lung metastases from metastatic breast cancer within the brain. In our previous studies, we investigated the AMT uptake kinetics in primary lung and breast cancers. Both of these cancer types, although heterogeneous themselves, showed substantial AMT tracer efflux out of the tumors at the primary tumor site, whereas metastases within the brain, similar to gliomas, had no significant tracer efflux. The majority of primary extracranial tumors studied until now had a peak tracer uptake within the first 25 minutes of the scan and showed decreasing uptake afterward. In contrast, gliomas and brain metastases typically showed prolonged, steadily increasing uptake beyond 30 minutes after tracer injection. Therefore, a different model was applied for the kinetic analyses of primary extracranial tumors than for brain tumors, and the absolute values obtained by these analyses are not directly comparable. However, one patient (22) included in the present study was also studied in the previous primary breast tumor study, along with histologic data from both the primary tumor and the brain metastasis. Both the primary and the metastatic tumor of this patient had an exceptionally high tryptophan uptake. Aside from this, the AMT kinetics in brain metastasis from this patient was generally more similar to GBMs than to the primary tumors from other patients.

It is important to mention that whereas the background AMT uptake in the lung or breast tissue was negligible, the AMT uptake of normal brain tissue was substantial because tryptophan is used for serotonin synthesis. Also, the blood-brain and blood-tumor barriers provide a more protected microenvironment in the brain, although these barriers are often compromised in high-grade tumors. The differences in AMT tracer kinetics at the extracranial versus the cerebral tumor sites suggest that the AMT uptake characteristics are affected by the tissue matrix the tumor cells are embedded in and not determined solely by the tumor type. Nevertheless, the results of the present study provide novel evidence that tumoral accumulation of AMT, measured by PET imaging, can be a useful imaging tool to differentiate between GBMs and common brain metastases in selected patients where such a distinction cannot be made on clinical grounds or conventional imaging.

We also found the AMT VD and tumor cortex/ratios to be the most accurate kinetic parameters to differentiate between lung and brain metastases. The same parameter also provided the highest PPV to differentiate GBMs from metastatic tumors. Tissue (including tumor) VD is high when the majority of injected radioactive tracer moves from the blood to the tissue (ie, high influx) and/or when only a small amount moves back from the tissue (tumor) to the blood (ie, low efflux). Thus, AMT VD is an indirect measure of net tryptophan transport from the blood to the tumor tissue, and its tumor/cortex ratio was found to be...
highly correlated with glioma proliferative activity in our recent study.\textsuperscript{31} Whether the observed VD differences among high-grade tumor types (GBM vs metastases; breast and lung cancer metastases) are related to tumor proliferation or other factors remains to be clarified in a larger series with histopathology comparisons. Also, we recently showed that patients with high-grade gliomas may have altered AMT kinetics in remote cortex,\textsuperscript{31} which could affect tumor/cortex ratios. In the present study, cortical AMT parameters of the reference cortical regions did not differ between the GBMs and metastases; therefore, it is likely that the observed AMT differences were driven by different tumoral AMT kinetics rather than by cortical changes.

### Differentiation from Nontumorous Lesions

Increased amino acid uptake is not confined to tumors but can occur in nontumorous lesions, most commonly associated with brain inflammation. Moderately increased methionine uptake on PET has been described in brain abscess, and a mild increase was also reported in active demyelinating lesions associated with multiple sclerosis.\textsuperscript{50–52}

Similarly, in preliminary studies, we observed focal increases of AMT uptake in a few patients with multiple sclerosis.\textsuperscript{53} The degree of these increases was mild/moderate, generally lower than increases seen in malignant brain tumors. Therefore, differentiation between high-grade brain tumors and demyelinating lesions may be possible by SUVs or differential tracer kinetics, but direct comparative studies are not yet available. Likewise, although increased AMT uptake can occur in nontumorous epileptic lesions (mostly seen in epileptogenic developmental brain malformations), AMT SUV lesion/cortex ratios in such regions rarely reach the high values observed in the present study.\textsuperscript{54} Nevertheless, there is some overlap between the AMT uptake seen in malignant brain tumors and some nontumorous lesions, and a firm differentiation of such lesions should be based on the history, clinical presentation, and all imaging findings rather than PET imaging alone.

### Limitations and Future Directions

Although the findings of this study are promising, further, prospective studies in larger patient populations using predefined analysis cutoff values are needed to confirm the differentiating accuracy of AMT-PET imaging. A larger study would allow multivariate analyses to test AMT-PET for its added clinical value compared to and in conjunction with MRI characteristics. It also remains to be determined if PET imaging with other amino acid radiotracers can provide similar or better accuracy for differentiating malignant gliomas from metastatic lesions as well as other, nontumorous lesions. Given that tumoral accumulation of several other amino acid PET tracers (eg, \([^{18}\text{F}]\)-labeled tyrosine, \([^{13}\text{C}]\)-methionine or \([^{18}\text{F}]\)-dopamine) is primarily driven by amino acid transport, quantification of tumoral uptake of these tracers could provide a similar distinction between GBMs and common metastatic brain tumors. The use of these PET tracers would increase the clinical impact of our observation because, currently, AMT has limited availability compared to some of the other amino acid PET tracers. This could change in the near future as recent advances in PET radiosynthesis, including the increasing availability and use of automated, modular PET radiosynthesis systems for routine synthesis of \([^{11}\text{C}]\)-labeled compounds,\textsuperscript{55,56} may facilitate the more widespread application of such radiotracers in the clinical setting. Development of an \([^{18}\text{F}]\)-labeled AMT analogue may also be possible. Nevertheless, such advanced imaging techniques are not expected to gain widespread clinical application for primary diagnosis of such lesions. Rather, AMT-PET imaging may be useful in selected patients with specific diagnostic dilemmas that cannot be resolved with current routine clinical imaging. For example, accurate pretreatment differentiation of some newly diagnosed ring-enhancing brain lesions may affect subsequent diagnostic steps (such as urgent brain biopsy or resection versus search for a primary tumor). In addition, because AMT is metabolized via the immunosuppressive kynurenine pathway, which is often upregulated in various cancers, clinical application of AMT-PET (or an \([^{18}\text{F}]\)-labeled AMT analogue) may be useful as novel enzyme inhibitors of this pathway enter clinical trials.\textsuperscript{58,57} Such drug trials can benefit from molecular imaging of tryptophan uptake and metabolism for patient screening and monitoring treatment effects.

### Acknowledgments

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Financial disclosure of reviewers: None reported.

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PAPER IV.

Increased tryptophan uptake on PET has strong independent prognostic value in patients with a previously treated high-grade glioma.

Kamson, D. O., Mittal, S., Robinette, N. L., Muzik, O., Kupsky, W. J., Barger, G. R., Juhász, C.

*Neuro Oncology*  
(Kamson et al., 2014)

PROBLEM

- Prognostication in previously treated high-grade gliomas is challenging
- Treatment related changes and recurrent glioma appear very similar on conventional MRI

GOAL

- To identify AMT-PET imaging markers that predict overall survival in previously treated high-grade glioma

APPROACH

- Various AMT-PET parameters were quantified in previously treated grade III-IV gliomas
- Patients were followed for at least one year or until they succumbed to their disease

FINDINGS

- Multiple AMT parameters predicted both 1-year and overall survival with high accuracy
- Survival prediction by AMT-PET was independent of age and tumor grade grade
- AMT-PET could further prognostic information in patients with disease progression indicated by T1-gad enhancement status change
Increased tryptophan uptake on PET has strong independent prognostic value in patients with a previously treated high-grade glioma

David O. Kamson, Sandeep Mittal, Natasha L. Robinette, Otto Muzik, William J. Kupsky, Geoffrey R. Barger, and Csaba Juha´sz

Background. Previously, we demonstrated the high accuracy of alpha-[11C]methyl-L-tryptophan (AMT) PET for differentiating recurrent gliomas from radiation injury. The present study evaluated the prognostic value of increased AMT uptake in patients with previously treated high-grade glioma.

Methods. AMT-PET was performed in 39 patients with suspected recurrence of World Health Organization grades III–IV glioma following surgical resection, radiation, and chemotherapy. Mean and maximum standardized uptake values (SUVs) and unidirectional AMT uptake (K) were measured in brain regions suspicious for tumor and compared with the contralateral cortex (ie, background). Optimal cutoff thresholds for 1-year survival prediction were determined for each AMT parameter and used for calculating the prognostic value of high (above threshold) versus low (below threshold) values for post-PET overall survival (OS).

Results. In univariate analyses, 1-year survival was strongly associated with 3 AMT parameters (SUV\textsubscript{max}, SUV\textsubscript{mean}, and tumor-to-background K-ratio; odds ratios: 21.3–25.6; \(P\leq .001\)) and with recent change in MRI contrast enhancement (odds ratio: 14.7; \(P = .02\)). Median OS was 876 days in the low- versus 177 days in the high-AMT groups (log-rank \(P < .001\)). In multivariate analyses, all 3 AMT parameters remained strong predictors of survival: high AMT values were associated with unfavorable 1-year survival (binary regression: \(P \leq .003\)) and shorter overall survival in the whole group (Cox regression hazard ratios: 5.3–10.0) and in patients with recent enhancement change on MRI as well (hazard ratios: 7.0–9.3; \(P \leq .001\)).

Conclusion. Increased AMT uptake on PET is highly prognostic for 1-year and overall survival, independent of MRI contrast enhancement and other prognostic factors in patients with a previously treated high-grade glioma.

Keywords: amino acid PET, high-grade glioma, MRI, survival, tryptophan.

High-grade gliomas have a dismal prognosis despite the survival benefit provided by microsurgical resection and subsequent radiation and chemotherapy. Long-term clinical management of this patient population can be very challenging. It is often difficult for the clinician to determine when to initiate additional treatment or redirect efforts toward palliative care. Clinical decision making is routinely guided by findings on serial conventional MRI, which includes T1-weighted gadolinium enhanced (T1-Gad) and T2-weighted or fluid attenuation inversion recovery (FLAIR) MR sequences. Generally, persistent enlargement or changing pattern of enhancement or increase in nonenhancing T2 or FLAIR signals is considered a sign of tumor recurrence or progression. The routine follow-up protocols for conventional MRI carry serious limitations. One of the greatest hindrances is the overlapping location and similar appearances of treatment-related necrosis and tumor recurrence. Discrepancies between the clinical and radiological status are also not uncommon. There are a handful of advanced MRI techniques, such as perfusion-weighted imaging (PWI), diffusion-weighted imaging (DWI), and MR spectroscopy, as well as PET imaging using amino acid and nucleotide radiotracers, that hold the promise of overcoming these limitations of conventional MRI. These newer techniques may help identify.

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residual tumor or differentiate radiation necrosis from recurrent neoplasm. However, the prognostic values of these imaging methods have yet to be investigated in depth.

In the present study, we utilized alpha-[14C]methyl-L-tryptophan (AMT) PET for prediction of survival. AMT is an amino acid radiotracer that is capable of tracking tumoral tryptophan transport and metabolism via the immunosuppres- sive kynurenine pathway. Earlier, we showed that both contrast-enhancing and nonenhancing gliomas have increased AMT uptake and that increased trapping in the glioma tissue is associated with the upregulation of indoleamine 2,3-dioxygenase (IDO), the rate-limiting enzyme of the kynurenine pathway. Detailed volumetric analysis has also demonstrated that the increased AMT uptake on PET is not confined to the area of contrast enhancement in high-grade gliomas but often extends to nonenhancing tissue, which was proven to have tumor cell infiltration in image-guided stereotactically acquired tissue samples. Furthermore, we have demonstrated that AMT-PET can differentiate radiation injury from tumor recurrence with a high degree of accuracy.

In the present study, we assessed the ability of AMT-PET to predict survival in patients with high-grade astrocytic tumors who had previously undergone microsurgical resection with subsequent radiation and chemotherapy. AMT-PET was performed due to the ambiguity of the information provided by conventional MRI. We hypothesized that higher degree of focal increase of AMT uptake in the previously treated cerebral hemisphere is associated with shorter survival. We also evaluated whether AMT-PET can provide added prognostic information to clinical and MRI data.

Materials and Methods

Patient Population

AMT-PET scans were acquired and analyzed from 39 patients (mean age, 57 y; range, 28–89; Table 1) (15 of these patients were included in our previous study on AMT-PET for differentiating recurrent gliomas from radiation injury14). The following inclusion criteria were used: (i) history of histologically proven high-grade (World Health Organization grade III or IV) astrocytic glioma; (ii) previous treatment with surgery (tumor resection or debulking) followed by radiation and chemotherapy; (iii) one of the following radiographic abnormalities: increasing size of contrast enhancement in high-grade gliomas but often extends to nonenhancing tissue, which was proven to have tumor cell infiltration in image-guided stereotactically acquired tissue samples. Furthermore, we have demonstrated that AMT-PET can differentiate radiation injury from tumor recurrence with a high degree of accuracy.

In the present study, we assessed the ability of AMT-PET to predict survival in patients with high-grade astrocytic tumors who had previously undergone microsurgical resection with subse- quent radiation and chemotherapy. AMT-PET was performed due to the ambiguity of the information provided by conventional MRI. We hypothesized that higher degree of focal increase of AMT uptake in the previously treated cerebral hemisphere is associated with shorter survival. We also evaluated whether AMT-PET can provide added prognostic information to clinical and MRI data.

MRI Protocol

Diagnostic MRIs with routine T2, FLAIR, and T1-Gad sequences acquired closest in time to the AMT-PET were used in this study. MRI was performed on either a Siemens MAGNETOM Trio TIM 3.0 Tesla scanner, a GE Signa HDxt 3.0 Tesla scanner, or a Philips Achieva TX 3.0 Tesla scanner, using similar parameters on all scanners.

PET Image Analysis

For image analysis, the 3D Slicer 3.6.3 software suite was used (http://www.slicer.org). On the AMT SUV images, regions of interest (ROIs) were drawn on suspicious (tumor and/or treatment affected) areas of increased AMT SUV in corresponding brain regions with contrast enhancement and/or T2/FLAIR signal changes on MRI (Fig. 1). In cases with no obvious AMT increases on visual assessment, ROIs were placed on brain tissue adjacent to the surgical cavity showing suspicious MRI abnormalities. As a reference (background) region, at least 3 ROIs were drawn on the homotopic cortex contralateral to the suspicious lesional area, and the values from these ROIs were averaged. Visual cortex, which has high physiologic AMT uptake, was not used as a background region. Using the AMT SUV image and K-values derived for patients who were alive at the end of the follow-up period (n = 12; mean follow-up time was 1066 d in these patients; Table 1). The study was approved by the Wayne State University Institutional Review Board with written informed consent obtained from all participants.

AMT-PET Scanning Protocol

The PET studies were performed using a Siemens EXACT/HR whole-body positron emission tomograph. The PET image in-plane resolution was 7.5 ± 0.4 mm at full-width half-maximum and 7.0 ± 0.5 mm at full-width half-maximum in the axial direction. The AMT tracer was synthesized by using a high-yield procedure as outlined previously. The procedure for AMT-PET scanning has also been described previously. In short, following 6 h of fasting, a slow bolus of AMT (3.7 MBq/kg) was injected over 2 min via a venous line. For collection of timed blood samples, a second venous line was established. In the ini- tial 20 min of the scan following tracer injection, a dynamic PET scan of the heart was performed to obtain the blood input function from the left cardiac ventricle noninvasively. The blood input function was continued beyond these initial 20 min by using venous blood samples (0.5 mL/sample, collected at 20, 30, 40, 50, and 60 min after AMT injection) as described previously. At 25 min after tracer injection, a dynamic emission scan of the brain (7 × 5 min) was obtained. Measured attenuation correction, scatter, and decay correction were applied to all PET images. For visualization of AMT uptake, averaged activity images 30–55 min postinjection were created and converted to an AMT standardized uptake value (SUV) image. For quantification of AMT accumula- tion, a Patlak graphical analysis was performed, which yielded AMT K-values, as described previously. The AMT K-value reflects the unidirectional uptake of tracer into tissue, which is proportional to the metabolism of tryptophan via the serotonin (in normal brain) and/or the kynurenine pathway.
from the Patlak plot, the following AMT-PET parameters were assessed: SUV_{mean} (the mean SUV in the ROI with the highest SUV), SUV_{max} (the maximal SUV measured in a single voxel within the area of suspicious imaging abnormality), SUV_{mean} tumor-to-background ratio, SUV_{max} tumor-to-background ratio, K_{mean} (the mean K-value in the suspicious lesion or area in the ROI with the highest K), and K_{mean} tumor-to-background ratio (the mean K-value in the ROI with the highest K divided by the mean K of the reference cortical ROIs). For the sake of simplicity, we will refer to the K_{mean} as K and to the K_{mean} tumor-to-background ratio as K-ratio.

**Table 1. Clinical data and MRI findings of the 39 high-grade glioma patients**

<table>
<thead>
<tr>
<th>#</th>
<th>Age/Gender</th>
<th>Grade</th>
<th>Follow-up, d</th>
<th>Alive</th>
<th>MRI Status</th>
<th>Days Between PET and...</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>First Surgery</td>
</tr>
<tr>
<td>1</td>
<td>28/F</td>
<td>III</td>
<td>371</td>
<td>Yes</td>
<td>T2/FLAIR change</td>
<td>393</td>
</tr>
<tr>
<td>2</td>
<td>32/M</td>
<td>III</td>
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<td>Enhancement change</td>
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<tr>
<td>3</td>
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<td>Enhancement change</td>
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<tr>
<td>4</td>
<td>50/M</td>
<td>III</td>
<td>904</td>
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<td>No or minimal enhancement</td>
<td>916</td>
</tr>
<tr>
<td>5</td>
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<td>III</td>
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<td>No or minimal enhancement</td>
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<td>10</td>
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</tr>
<tr>
<td>11</td>
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<td>2355</td>
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<tr>
<td>12</td>
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<tr>
<td>13</td>
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</tr>
<tr>
<td>14</td>
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<td>IV</td>
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<tr>
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<td>T2/FLAIR change</td>
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<tr>
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<td>Enhancement change</td>
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<tr>
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<td>Stable enhancement</td>
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</tr>
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<td>188</td>
</tr>
<tr>
<td>34</td>
<td>69/M</td>
<td>IV</td>
<td>94</td>
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<td>Enhancement change</td>
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</tr>
<tr>
<td>35</td>
<td>73/M</td>
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<td>Enhancement change</td>
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<td>440</td>
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<td>Enhancement change</td>
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</tr>
<tr>
<td>37</td>
<td>79/M</td>
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<td>Enhancement change</td>
<td>618</td>
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<tr>
<td>38</td>
<td>81/M</td>
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<td>Enhancement change</td>
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<td>39</td>
<td>89/F</td>
<td>IV</td>
<td>257</td>
<td>No</td>
<td>Enhancement change</td>
<td>234</td>
</tr>
</tbody>
</table>

No or minimal enhancement: complete lack of abnormal enhancement or presence of enhancement <5 mm bidimensionally.

Statistical Analysis

First, a receiver operating characteristic (ROC) analysis was performed, and areas under the curve (AUCs) were used to determine the most accurate AMT-PET–related variable(s) and an optimal cutoff threshold value (with highest sensitivity plus specificity) to predict 1-year survival. These threshold values were then applied to categorize the values of each AMT measure as either “high” or “low” to be used for the analysis of overall survival (OS) after the PET scan.

Survival time distribution among the high and low value groups of each AMT measure, as defined by the ROC analyses,
was examined using the Mantel–Cox log-rank test. First, univariate analyses were performed to explore the association of survival and potential clinical and MRI prognostic factors (age, gender, World Health Organization grade of tumor, a second surgery or bevacizumab treatment after AMT-PET, as well as enhancement change on T1-Gad [as defined in the Patient Population section] within 6 mo preceding AMT-PET) and AMT parameters. Prognostic factors found significant in the univariate analysis along with factors reported to be strongly prognostic by the literature (such as age and histologic grade)28–30 were included in multivariate analyses with each significant AMT parameter. In both the univariate and multivariate analyses, binary logistic regression was used to obtain odds ratios (ORs) for 1-year survival, and the proportional hazard Cox regression was utilized to obtain hazard ratios (HRs) for OS.

Cox regression survival analyses were also performed to test the ability of the various AMT-PET parameters to predict survival. Based on the results of these comparisons and in continuity with our previous studies,14,19,21,23,31 we have restricted the Results section to the presentation of the 3 best performing PET parameters: the SUVmean, SUVmax, and K-ratio. From here on, we will refer to these as the studied AMT parameters. Results for the other parameters (SUV tumor-to-background ratios and tumor K-values), which showed poorer performance, are not shown.

Finally, because of the strong prognostic association, survival analyses were repeated in a subgroup of patients with enhancement change on T1-Gad (ie, increasing size of contrast enhancement or the appearance of a new enhancing lesion) suspicious for tumor progression on MRI within 6 months before AMT-PET (n = 29). Statistical analysis was carried out using SPSS Statistics 20.0 software. P < .05 was considered statistically significant.

### Results

#### Prediction of Survival by AMT-PET in the Whole Group

The estimated median survival time was 417 days (95% confidence interval [CI]: 245–589 d), that is, 13.9 months after the AMT-PET scan and 27.4 months from the initial surgery. Of the 39 patients, 28 (72%) were alive 6 months, 20 (51%) 1 year, and 8 (25%) 2 years after the AMT-PET scan. The longest survivor was still alive more than 6 years after the PET scan.

For prediction of 1-year survival, the ROC analyses showed high AUCs (0.83–0.86) with all studied AMT parameters. SUVmean had the highest sensitivity with 89%, while SUVmax had the highest specificity (80%) among the SUV-based parameters. The K-ratio also showed high specificity (88%; Table 2). Estimated median survival times were significantly different between the high and low groups in all AMT measures on the log-rank tests (P < .001). Values deemed as low (ie, below threshold, as defined by ROC analysis) were associated with ~5-fold longer OS time (876 d for low-uptake groups of each AMT parameter vs 177 d for high-uptake groups; Table 3, Fig. 2).

In univariate analyses, 1-year survival was significantly associated with SUVmean (OR: 25.6), SUVmax (OR: 21.3), and K-ratio (OR: 24.4) (P ≤ .001 for all), as well as with recent change in
Table 2. Results from the ROC analysis of 1-y survival prediction in the whole population (n = 39) and in a patient subgroup (n = 29) with recent enhancement change (within 6 mo) on follow-up MRI

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Whole Population</th>
<th>Patients With Recent Enhancement Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUV&lt;sub&gt;mean&lt;/sub&gt;</td>
<td>19/20 3.88 0.86 89% 75%</td>
<td>18/11 3.88 0.83 89% 73%</td>
</tr>
<tr>
<td>SUV&lt;sub&gt;max&lt;/sub&gt;</td>
<td>19/20 4.62 0.83 84% 80%</td>
<td>18/11 4.36 0.77 89% 73%</td>
</tr>
<tr>
<td>K-ratio</td>
<td>17/17 1.63 0.86 76% 88%</td>
<td>16/10 1.63 0.87 75% 90%</td>
</tr>
<tr>
<td>MRI enhancement change</td>
<td>19/20 Yes/no 0.70 95% 45%</td>
<td>– – – – – – – –</td>
</tr>
</tbody>
</table>

Abbreviations: N (d/a), number of patients deceased/alive within 1 y; Sens, sensitivity; Spec, specificity. Threshold: most appropriate threshold found for prediction of survival or death within 1 y. AUC as indicated by ROC curve.

Table 3. Results of the log-rank analyses for the dichotomized groups (high vs low uptake, based on the ROC analysis) of different AMT parameters

<table>
<thead>
<tr>
<th>AMT Parameter</th>
<th>Whole Population (n = 39)</th>
<th>Recent Enhancement Change (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimated Median Survival Time, d</td>
<td>N, h/l P</td>
</tr>
<tr>
<td>SUV&lt;sub&gt;mean&lt;/sub&gt;</td>
<td>High (95% CI) 177 (137–217)</td>
<td>876 (508–1244) 22/17 &lt;.001</td>
</tr>
<tr>
<td>SUV&lt;sub&gt;max&lt;/sub&gt;</td>
<td>High (95% CI) 177 (140–214)</td>
<td>876 (450–1302) 20/19 &lt;.001</td>
</tr>
<tr>
<td>K-ratio</td>
<td>High (95% CI) 177 (154–200)</td>
<td>876 (380–1372) 15/19 &lt;.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimated Median Survival Time, d</th>
<th>N, h/l P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUV&lt;sub&gt;mean&lt;/sub&gt;</td>
<td>High (95% CI) 177 (137–217)</td>
<td>876 (508–1244) 22/17 &lt;.001</td>
</tr>
<tr>
<td>SUV&lt;sub&gt;max&lt;/sub&gt;</td>
<td>High (95% CI) 177 (140–214)</td>
<td>876 (450–1302) 20/19 &lt;.001</td>
</tr>
<tr>
<td>K-ratio</td>
<td>High (95% CI) 177 (154–200)</td>
<td>876 (380–1372) 15/19 &lt;.001</td>
</tr>
</tbody>
</table>

Enhancement on MRI (OR: 14.7, P = .001), but not with other potential prognostic factors (Table 4). Univariate analyses of the same variables with OS also showed similar results, with high values in AMT parameters associated with up to 10-fold hazard (P < .001 for all 3 AMT parameters; Table 4), while recent enhancement change on MRI was associated with ~9-fold hazard (P = .003; Table 4). Repeat surgery (16 patients) and/or bevacizumab treatment (16 patients, including 5 who also had repeated surgery) after AMT-PET were not prognostic (Table 4).

In multivariate analyses with each studied AMT parameter, including age, histologic grade, and (based on the results of the univariate analyses) recent enhancement change as covariates, AMT SUV<sub>mean</sub>, SUV<sub>max</sub>, and K-ratio were all significant independent predictors of 1-year survival (ORs: 18.5–35.7, P ≤ .003; Table 5), while recent enhancement change reached significance only in the analysis with SUV<sub>mean</sub> (OR: 14.3, P = .04; Table 5; see examples in Fig. 3). Using the same covariates, SUV<sub>mean</sub>, SUV<sub>max</sub>, and K-ratio were also independent predictors of OS (HRs: 5.3–10.0, P ≤ .001 for all), and recent enhancement change was significant in all analyses as well (HRs: 5.6–11.4, P ≤ .03; Table 5).

#### Prediction of Survival in the Subgroup With Recent MRI Enhancement Change

The ROC analyses for 1-year survival prediction indicated high AUCs (0.77–0.87) for all studied AMT parameters in the subgroup with recent MRI enhancement change. Threshold values were identical to those of the whole patient population except for SUV<sub>max</sub>, which was slightly lower in this patient subgroup (Table 2). The estimated median survival was ~3.5 times longer in the low-uptake groups defined by the AMT parameters (log-rank P ≤ .001 in all; Fig. 2). For 1-year survival, the age- and grade-adjusted multivariate analyses of separate AMT parameters again found all 3 AMT parameters to be significant independent predictors (K-ratio OR: 30.3, 95% CI: 2.6–333, P = .006; SUV<sub>mean</sub> OR: 23.8, 95% CI: 3.0–200, P = .003; SUV<sub>max</sub> OR: 14.5, 95% CI: 2.2–100, P = .005). The age- and grade-adjusted OS prediction also showed similar results to the whole population, with K-ratio (HR: 9.3, 95% CI: 2.7–32.3, P ≤ .0005), SUV<sub>mean</sub> (HR: 7.0, 95% CI: 2.2–22.2, P = .001), and SUV<sub>max</sub> (HR: 7.0, 95% CI: 2.2–22.2, P = .001) being highly significant predictors.

#### Discussion

The main finding of this study is that increased AMT uptake measured by PET is a very strong predictor of 1-year and overall survival in patients with previously treated high-grade gliomas. Importantly, the studied PET parameters showed a strong prognostic value independently of enhancement changes on MRI and independently of several other potential clinical and MRI prognostic factors. Since SUVs showed excellent prognostic performance (similar to AMT K-ratios, which require dynamic image acquisition...
and blood input function), the results strongly indicate that a single time-point static posttreatment AMT-PET scan could provide valuable prognostic information to supplement conventional MRI.

**Univariate Analysis of Prognostic Factors**

<table>
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<tr>
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<th>1-y Survival</th>
<th>OS</th>
</tr>
</thead>
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<tr>
<td></td>
<td>OR (95% CI)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Age</td>
<td>0.98 (0.9–1.0)</td>
<td>0.98 (0.95–1.0)</td>
</tr>
<tr>
<td>Gender</td>
<td>0.87 (0.2–3.2)</td>
<td>0.71 (0.32–1.6)</td>
</tr>
<tr>
<td>Changing enhancement on MRI, yes/no</td>
<td>14.7 (1.6–125)</td>
<td>9.1 (2.1–33.3)</td>
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<tr>
<td>Bevacizumab after AMT-PET, yes/no</td>
<td>1.7 (0.45–6.6)</td>
<td>1.6 (0.70–3.5)</td>
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<tr>
<td>Surgery after AMT-PET, yes/no</td>
<td>0.89 (0.25–3.2)</td>
<td>1.1 (0.49–2.3)</td>
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<td>Histologic grade, III vs IV</td>
<td>6.0 (0.6–55.5)</td>
<td>3.3 (0.78–14.2)</td>
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<tr>
<td>SUV&lt;sub&gt;mean&lt;/sub&gt;, high vs low</td>
<td>25.6 (4.2–142)</td>
<td>4.8 (2.0–11.1)</td>
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<tr>
<td>SUV&lt;sub&gt;max&lt;/sub&gt;, high vs low</td>
<td>21.3 (4.0–111)</td>
<td>6.3 (2.5–16.6)</td>
</tr>
<tr>
<td>K-ratio, high vs low</td>
<td>24.4 (3.8–166)</td>
<td>10.0 (3.2–33.3)</td>
</tr>
</tbody>
</table>

*Significant.

**Advanced MRI and PET in Posttreatment Glioma Prognosis**

Current clinical follow-up protocols mainly look for changes in the enhancing and/or the T2/FLAIR hyperintense volume to...
determine disease progression or response to therapy in patients with treated malignant gliomas.

As shown by others and corroborated by our study as well, contrast enhancement changes on MRI are prognostically significant. However, such changes can be caused by therapy-induced tissue damage (e.g., radiation necrosis), tumor recurrence, or, commonly, a combination of

Table 5. Multivariate analyses of prognostic factors for 1-y survival (binary regression analysis) and for OS (Cox proportional hazard regression analysis) after AMT-PET, with separate analyses performed involving SUV\text{mean}, SUV\text{max}, and K-ratio AMT parameters

<table>
<thead>
<tr>
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<th>SUV\text{max}</th>
<th>K-ratio</th>
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<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
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<tr>
<td>AMT-PET, high vs low</td>
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<td>18.5 (2.9–125)</td>
<td>35.7 (3.3–333)</td>
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<tr>
<td>Age</td>
<td>1.0 (0.95–1.09)</td>
<td>1.0 (0.95–1.10)</td>
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<td>Histologic grade, III vs IV</td>
<td>2.0 (0.04–111)</td>
<td>3.2 (0.08–143)</td>
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<tr>
<td>Changing enhancement, yes vs no</td>
<td>14.3 (1.07–200)</td>
<td>9.1 (0.71–111)</td>
<td>17.9 (0.78–500)</td>
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Multivariate Analysis of OS

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<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>AMT-PET, high vs low</td>
<td>5.9 (2.2–16.7)</td>
<td>5.3 (1.96–16.7)</td>
<td>10.0 (2.9–33.3)</td>
</tr>
<tr>
<td>Age</td>
<td>0.99 (0.97–1.03)</td>
<td>1.00 (0.97–1.03)</td>
<td>0.99 (0.96–1.02)</td>
</tr>
<tr>
<td>Histologic grade, III vs IV</td>
<td>0.77 (0.15–4.0)</td>
<td>0.93 (0.18–5.0)</td>
<td>1.05 (0.20–5.6)</td>
</tr>
<tr>
<td>Changing enhancement, yes vs no</td>
<td>11.4 (2.4–100)</td>
<td>7.5 (1.61–50.0)</td>
<td>5.6 (1.18–25.0)</td>
</tr>
</tbody>
</table>

*Significant.

Fig. 3. Coregistered T1-Gad, AMT-PET, and MRI/PET fusion images of 2 patients with a contrast-enhancing lesion in the right hemisphere, suspicious for glioblastoma recurrence. (A) Patient #25 had low AMT uptake in this lesion (SUV\text{max}: 4.18; K-ratio: 1.30) and survived for almost 3 years after the PET scan. (B) Patient #9 showed very high AMT uptake (SUV\text{max}: 5.04; K-ratio: 2.17) and died within 6 months after AMT-PET.
both.\textsuperscript{6–8} Additionally, patients often present with a conflicting radiological and clinical status, showing worsening disease in one and stable status in the other. The situation is further complicated by the use of antiangiogenic agents, such as bevacizumab, which may mask progressive disease by temporarily decreasing enhancement on MRI.\textsuperscript{20–22} Thus, there is a definite need for imaging modalities of superior prognostic value. Advanced MRI involving PWI, DWI, and MR spectroscopy, as well as PET imaging utilizing glucose, amino acid, and nucleotide radiotracers, were shown to distinguish radiation necrosis from recurrent tumor and may be used to overcome the prognostic limitations of conventional MRI.\textsuperscript{8–14}

Prognostic MRI studies mainly utilized PWI to investigate blood volume or DWI to assess the apparent diffusion coefficient in the areas suspicious for tumor recurrence.\textsuperscript{30,32,35,36} PET studies of the same kind utilized \textsuperscript{[18]F}fluoro-deoxy-D-glucose (FDG), a measure of glucose metabolism; \textsuperscript{[18]F}fluoro-L-thymidine (FLT), a marker of tumoral proliferative activity; or amino acid tracers such as \textsuperscript{[11]C}methyl-L-methionine (MET), \textsuperscript{[18]F}fluoro-ethyl-tyrosine (FET), \textsuperscript{[18]F}fluoro-dehydroxy-phenylalanine (FDOPA), and AMT, which measure amino acid transport and/or metabolism in tumor tissue. While all these amino acid PET tracers are predominantly measure amino acid transport and/or metabolism in tumor tissue, the kynurenine pathway, which is commonly upregulated in brain AMT uptake and trapping are partly driven by metabolism of an enzyme that can be present in some tumors), MET and AMT (although FDOPA is a substrate of aromatic acid decarboxylase, ent. In contrast to FET and FDOPA, which are not metabolized or cells following transport. AMT uptake and trapping are partly driven by metabolism via IDO, the rate-limiting enzyme of the immunosuppressive kynurenine pathway, which is commonly upregulated in brain tumors.\textsuperscript{17,20,39,60} IDO-mediated tryptophan depletion and accumulation of toxic kynurenine metabolites may lead to proliferation arrest of tumor-invading cytotoxic T-lymphocytes, inhibiting a major mechanism of antitumoral immune response.\textsuperscript{17,18} IDO expression has also been linked to worse survival in glioblastomas.\textsuperscript{39,41} These data, at least in part, could explain the strong prognostic value of in vivo uptake of AMT, which is a substrate of IDO due to the low specificity of this enzyme. Inhibition of the kynurenine pathway is a potential target for potent IDO inhibitors,\textsuperscript{30–32} and AMT-PET may be utilized to identify patients who could potentially benefit from such new medications in future trials.

The few PET studies that utilized a single time-point posttreatment scan, similar to our current one, reported mixed results in terms of prognostic value. Van Laere et al.,\textsuperscript{33} using FDG and MET in a posttreatment mixed low- and high-grade glioma population, found the uptake of both tracers to be predictive of OS both individually and in combination. However, tumor histologic grade was not included in the multivariate analysis, and no separate survival analysis was performed with high-grade gliomas.\textsuperscript{43} A subsequent study using the same combination of PET tracers could not reproduce these findings in a pure glioblastoma population,\textsuperscript{44} while Cayssens et al.\textsuperscript{45} also did not find MET SUV\textsubscript{max} to be predictive of OS in posttreatment gliomas. Nariai et al.,\textsuperscript{46} measuring MET uptake in a large glioma population, reported the lack of residual MET-PET uptake after initial treatment (resection and radiation) to be associated with better OS; however, their subgroup without residual MET uptake comprised only 8 patients versus the 27 patients with MET positive remnants. Also, they did not perform multivariate analysis involving conventional MRI parameters; neither did they report the time frame in which MET-PET was performed after the initial therapy. Therefore, their findings are difficult to compare to the results of our current study.

The majority of previous PET and advanced MRI studies that investigated OS utilized a dual (or multi) time-point approach acquiring one scan before and another during or after treatment. These studies often generated parametric response maps (PRMs) based on voxel-wise perfusion, diffusion, or radiotracer uptake changes between time points within the regions suspicious for the presence of tumor.\textsuperscript{8,30,36} PRMs can be used for early stratification of responders versus nonresponders to a specific therapy. This prognostic information is quite different from that of our current study, which provides indirect information on response to therapy, yet represents a clinically more feasible single time-point approach. Perfusion and/or diffusion PRMs on MRI between the pre- and intratreatment scans were found to be predictive of OS.\textsuperscript{30,32,35,36} In the assessment of response to radiation, PET-FET showed reduction of the high-uptake volume to be associated with longer OS.\textsuperscript{47} Since the reference images in all of these studies were acquired from treatment-naïve patients, their findings are not comparable to ours. Another set of PET studies that utilized the same dual time-point approach investigated response to bevacizumab therapy in recurrent high-grade gliomas. Decrease in the high FET uptake volume was also associated with an OS benefit in this setting: FET responders lived 20 months longer than nonresponders.\textsuperscript{48} PRMs of regional FLT uptake decreases were associated with better 1-year survival as well as with a 7- to 8.7-month OS benefit after bevacizumab treatment.\textsuperscript{13,69,50} These findings are more modest than our 23.3-month OS benefit in the entire population with low AMT uptake, or the 13.5 months in patients with recent enhancement change. According to one study, changes in FLT uptake kinetics could predict 1-year survival with 100% accuracy, but the study had only 6 patients surviving longer than 1 year after bevacizumab treatment.\textsuperscript{45} Another study reported the lack of FLT reduction 6 weeks after bevacizumab treatment to be an independent negative predictor of OS, with HRs of 10.1 in multivariate and 7.9 in univariate analyses.\textsuperscript{45} In our current cohort, the K-ratio predicted OS with a similar HR value (10.0). Lastly, a recent study using FLT and FDOPA PRMs in bevacizumab treatment response assessment showed that although both radiotracers could predict 6-month survival, only FDOPA predicted OS; however, the arms of the Kaplan–Meier curve met at the 400th day.\textsuperscript{46} None of these above-mentioned studies reported any single time-point parameter that could predict OS.

Potential Implications of the Findings

As mentioned above, our study aimed to investigate the prognostic value of AMT-PET in a clinically broader sense, not restricted to response assessment for a certain kind of therapy. The present paper is one of the few imaging studies that found a single time-point study to be highly predictive of OS in posttreatment high-grade gliomas. In fact, we found a multitude of AMT parameters to have similarly strong prognostic value. The most clinically feasible and practical parameter was SUV\textsubscript{max}, which can be assessed semiautomatically without the need for any additional measurements or procedures such as blood radioactivity sampling, which is required for the estimation of K. The prognostic
information provided by AMT-PET could greatly clarify disease status and associated prognosis, even in the presence of treatment-induced changes that make conventional MRI findings difficult to interpret (as illustrated in Fig. 3). Based on our study, the potential clinical value of AMT-PET is particularly high in patients with MRI contrast enhancement suspicious for tumor progression. In our cohort, changing contrast enhancement was associated with survival ranging from 14 to 2498 days, while AMT uptake was a strong predictor in this subgroup. Compared with dual time-point prognostic studies, a single posttreatment AMT-PET could be easily incorporated into the clinical follow-up at any time point after suspected progression on conventional MRI, without prior enrollment in a study; thus, it might be a cost-efficient tool to determine when to start or stop antiangiogenic or other rescue therapy. Comparative studies have yet to be performed to investigate whether our present findings are specific to AMT, or can be achieved using other amino acid tracers and to test how AMT-PET can complement advanced MRI techniques in the posttreatment imaging of malignant gliomas.

Limitations

Our studied population is likely biased toward cases with better outcomes, since patients with very severe early posttreatment progression did not survive long enough to reach the eligibility period of this study, and patients in severe condition requiring urgent intervention were excluded from the PET studies (see Materials and Methods). Although our cohort may not be completely representative of the general high-grade glioma population, it does represent patients for whom prognostic imaging was highly relevant. There was some variability in the treatment that patients received in their later disease course—for instance, some received bevacizumab around the AMT-PET scan or had a second surgery. However, neither of these factors turned out to be a significant predictor of survival. Also, a recently concluded large bevacizumab trial suggests that this drug does not have a robust effect on OS.51 Tumor recurrence suggested by MRI was not corroborated by histologic data in a sufficient number of cases to provide meaningful information on progression-free survival, at least not beyond the findings of our recent study on radiation necrosis versus glioma recurrence.16 Besides, although OS is a relatively crude measure of outcome that ignores quality of life, it is still the most reliable, least ambiguous, and easiest to interpret outcome measure for both patients and clinicians. Throughout the imaging literature, clinical, radiographic and histologic disease progression are defined in different ways while our results emphasize that progression assessment on conventional MRI can be greatly enhanced by AMT-PET in terms of both 1-year and overall survival prediction.

AMT is a carbon-11-labeled radiotracer with a short (20 min) half-life requiring an on-site cyclotron for its synthesis, restricting the availability of AMT to only a few centers currently. It has yet to be determined how specific our observations are to AMT and whether similar strong results can be achieved with other PET radiotracers. Still, these findings do support the clinical usefulness of amino acid radiotracers in posttreatment glioma imaging and might encourage other centers to test and compare other amino acid PET probes for these applications.

Conclusion

Several AMT-PET parameters, including a simple SUV measure, were found to be strongly predictive of OS in posttreatment high-grade astrocytic gliomas. The prognostic value of AMT-PET was independent of age, histologic grade, or recent changes in contrast enhancement on MRI. Therefore, AMT-PET can be a useful imaging tool to supplement clinical follow-up imaging in conjunction with conventional MRI in patients with previously treated malignant gliomas.

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Conflict of interest statement. None declared.

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