Accurate Delineation of Glioma Infiltration by Advanced PET/MR Neuro-Imaging (FRONTIER Study): A Diagnostic Study Protocol

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BACKGROUND: Glioma imaging, used for diagnostics, treatment planning, and follow-up, is currently based on standard magnetic resonance imaging (MRI) modalities (T1 contrast-enhancement for gadolinium-enhancing gliomas and T2 fluid-attenuated inversion recovery hyperintensity for nonenhancing gliomas). The diagnostic accuracy of these techniques for the delineation of gliomas is suboptimal.

OBJECTIVE: To assess the diagnostic accuracy of advanced neuroimaging compared with standard MRI modalities for the detection of diffuse glioma infiltration within the brain.

METHODS: A monocenter, prospective, diagnostic observational study in adult patients with a newly diagnosed, diffuse infiltrative glioma undergoing resective glioma surgery. Forty patients will be recruited in 3 years. Advanced neuroimaging will be added to the standard preoperative MRI. Serial neuronavigated biopsies in and around the glioma boundaries, obtained immediately preceding resective surgery, will provide histopathologic and molecular characteristics of the regions of interest, enabling comparison with quantitative measurements in the imaging modalities at the same biopsy sites.

DISCUSSION: In this clinical study, we determine the diagnostic accuracy of advanced imaging in addition to standard MRI to delineate glioma. The results of our study can be valuable for the development of an improved standard imaging protocol for glioma treatment.

EXPECTED OUTCOME: We hypothesize that a combination of positron emission tomography, MR spectroscopy, and standard MRI will have a superior accuracy for glioma delineation compared with standard MRI alone. In addition, we anticipate that advanced imaging will correlate with the histopathologic and molecular characteristics of glioma.

KEYWORDS: Delineation, Diagnostic accuracy, Glioma, MRI, MRS, PET, Study protocol

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RATIONALE AND BACKGROUND INFORMATION

Gliomas represent 80% to 90% of parenchymal brain tumors in adults with an incidence of 5.9 per 100 000 person-years; approximately 1000 patients per year in the Netherlands.[1]

ABBREVIATIONS: CHO, [11C-]Choline; **CRF,** case report forms; **FET,** [18F-]Fluoroethyl-tyrosine; **FLAIR,** fluid-attenuated inversion recovery; **METC,** Medical Ethical Committee; **MRS,** magnetic resonance spectroscopy; **PET,** positron emission tomography; **VUmc,** VU University Medical Center

Most gliomas show extensive infiltration in the brain parenchyma. These so-called diffuse gliomas universally recur without exception, resulting in death despite standard treatment, which consists of an as extensive as possible resection followed by radiation and chemotherapy.

Both resective surgery and adjuvant radiation therapy are based on T1 contrast-enhancement for gadolinium-enhancing gliomas and on T2 fluid-attenuated inversion recovery (FLAIR) hyperintensity volume outlines for nonenhancing gliomas. This strategy is founded on early and preliminary observations and has remained unchanged since.[2,3] Diffuse gliomas recur locally in the vast

majority of patients, even after seemingly radical surgical removal and radiation therapy with 2 cm margins. This, and the fact that glioma infiltration has been demonstrated to extend up to 2 cm beyond standard magnetic resonance imaging (MRI) outlines, underscores that, up until now, delineation of these neoplasms has been less than optimal.[4-7]

Several publications provide arguments for underestimation of the spread of diffuse gliomas using standard MRI and potential benefit from advanced MRI and positron emission tomography (PET) imaging. Advanced imaging, such as diffusion-weighted imaging, perfusion-weighted imaging, magnetic resonance spectroscopy (MRS), and PET, has been shown to be able to identify tumor in areas of normal standard MRI signal.[8-10]

Our study addresses a clinically relevant research question that so far has not been answered adequately: What is the best neuroimaging approach to discriminate areas with glioma infiltration from brain tissue without glioma cells?

STUDY GOALS AND OBJECTIVES

The goal of this study is, thus, to determine the best neuroimaging approach for glioma delineation. The specific objectives are:

- To assess the increase in diagnostic accuracy of adding advanced neuroimaging modalities to standard MRI for the detection of diffuse glioma infiltration within the brain.
- To correlate the information obtained by standard and advanced imaging to histologic and molecular characteristics of the tissue.

We hypothesize that advanced neuroimaging, in combination with standard MRI, will have a superior diagnostic accuracy in comparison with standard MRI alone. In addition, we hypothesize that histologic and molecular characteristics of (different areas of) glioma will correlate better with advanced imaging than with standard imaging.

STUDY DESIGN

The study design is a monocenter, prospective, diagnostic observational study.

METHODOLOGY

Subjects

Inclusion Criteria

Included patients will be 18 years and older with an MRI interpretation of a diffuse glioma by an experienced neuroradiologist and who have an indication for resective surgery; the indication will be confirmed by the multidisciplinary neuro-oncology tumor board.

Exclusion Criteria

Patients who are pregnant or have undergone previous brain surgery, cranial irradiation, or chemotherapy will be excluded. Patients with other brain pathology on MRI, such as stroke or multiple sclerosis also will be excluded as well as patients with a tumor located infratentorially or in the spinal cord.

Withdrawal Criteria

Patients who do not successfully undergo 1 PET scan will be withdrawn from the study. A summary of all criteria is given in Table 1.

Study Description

The study is separated into 2 phases (Figure). In both phases, standard and advanced imaging will be performed preoperatively (Table 2). Immediately preceding resective surgery, serial image-guided neuronavigated biopsies in and around the glioma boundaries will be obtained using a stereotactic drilling technique.[11] Two samples will be collected from each biopsy location: 1 for assessment of histopathologic characteristics and 1 for molecular analysis.

Phase I is designed to decide on the optimal PET tracer, to simplify PET scanning methodology, and to develop a robust MRI protocol for glioma volume estimation. Eight patients will receive a dynamic PET protocol with invasive blood sampling and image-derived carotid input function for metabolite analysis of [18F-] Fluoroethyl-tyrosine (FET) and [11C-]Choline (CHO) tracers, as well as advanced MR imaging. The data obtained will be used to establish a simplified PET protocol and to determine which of both PET tracers will be further pursued in the next study phase.

To obtain a total sample size of 20 patients with a high-grade glioma (WHO grade III or IV) and 20 with a low-grade glioma

TABLE 1. Inclusion/Exclusion/Withdrawal Criteria[a]

Inclusion Criteria	Exclusion Criteria	Withdrawal Criteria
Adult (18 y and older)	Previous brain surgery, cranial radiotherapy, or chemotherapy	Not successfully undergoing 1 PET scan
MRI interpretation of diffuse glioma by an experienced neuroradiologist	Other brain pathology on MRI, such as infarction or multiple sclerosis	
Indication for resective surgery confirmed by the multidisciplinary neuro-oncology workgroup	Tumor located infratentorially or in the spinal cord	
	Pregnancy	

[a]MRI, magnetic resonance imaging; PET, positron emission tomography.

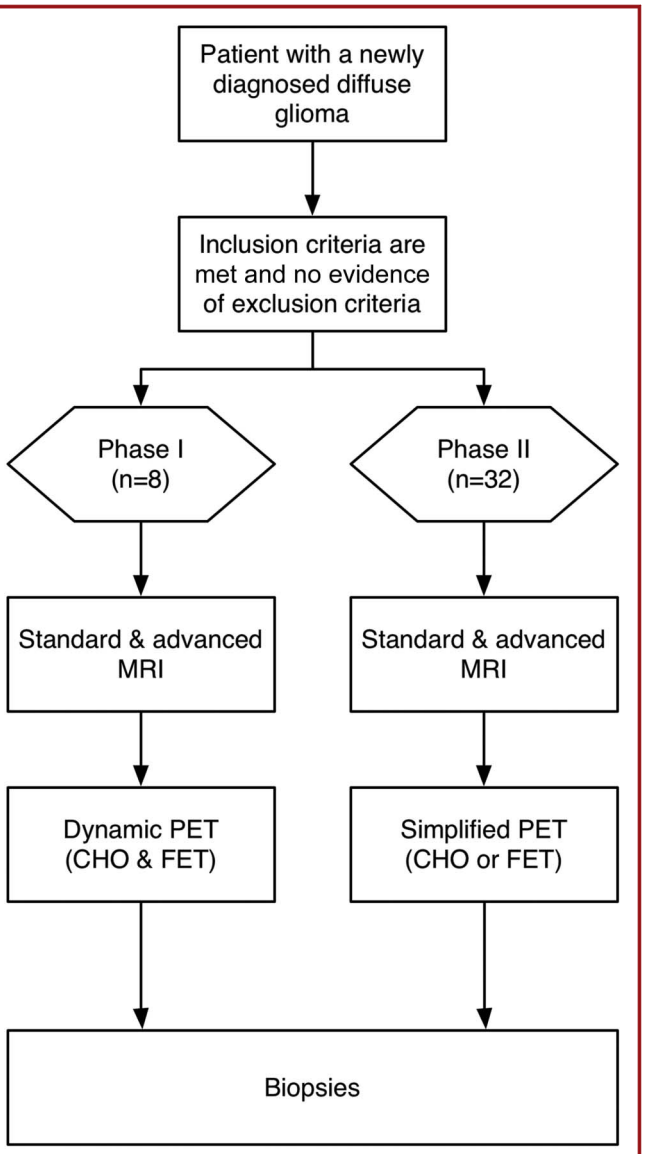

FIGURE. *Imaging protocol for different phases of the study. Cho, 11C-Choline; FET, [18F]Fluoroethyl-tyrosine; MRI, magnetic resonance imaging; PET, positron emission tomography.*

(WHO grade II), 20 additional patients will receive single advanced MRI and selected simplified PET imaging in the second phase to complete the data acquisition according to the sample size calculation for the main research question.

Outcome Measures

MRI

MRI will be performed using the Philips Achieva whole-body 3.0T MR-scanner, equipped with the standard head coil. Table 2 shows the different techniques.

TABLE 2. Quantitative Imaging Parameters[a]

Modality/ Technique	Parameter 1	Parameter 2	Parameter 3
Standard MRI			
2D T1	T/N ratio		
2D T2	T/N ratio		
3D FLAIR	T/N ratio		
2D T1 after contrast	T/N ratio		
3D T1 after contrast	T/N ratio		
Advanced MRI			
3D MRS	T/N ratio	Cho/NAA ratio	
ASL	T/N ratio	Relative CBF	
DTI	T/N ratio	FA	ADC
DSC	T/N ratio	Relative CBV	Relative CBF
PET			
11C-Choline	T/N ratio	SUV	Net influx rate
[18F]Fluoroethyl-tyrosine	T/N ratio	SUV	Net influx rate

[a]2D, 2-dimensional; 3D, 3-dimensional; ADC, apparent diffusion coefficient; ASL, arterial spin labeling; CBF, cerebral blood flow; CBV, cerebral blood volume; Cho, choline; DSC, dynamic susceptibility contrast; DTI, diffusion tensor imaging; FA, fractional anisotropy; FLAIR, fluid-attenuated inversion recovery; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NAA, N-acetyl aspartate; PET, positron emission tomography; SUV, standardized uptake value; T/N ratio, tumor-to-normal radioactivity (PET) or signal intensity (MRI).

PET

PET will be performed using the Philips Gemini time-of-flight PET-CT scanner or the Philips Ingenuity time-of-flight PET/MRI-scanner. After intravenous administration of 370 megabecquerel of $[^{15}O]H_2O$, a 10-minute dynamic scan will be acquired. This is followed by a 40-minute dynamic scan after injection of 200 megabecquerel CHO. With a minimum of 4 hours after injection of CHO, the FET scan will be performed the same day, using 200 megabecquerel FET and a scan time of 90 minutes. During the scans, manual blood samples will be withdrawn in order to calibrate the online collected arterial input functions and to derive a fully metabolite-corrected plasma input function.

Of each biopsy site, qualitative (high, normal, or low signal) and quantitative parameters will be acquired by an experienced neuroradiologist and a nuclear medicine physician (Table 2).

Pathology

Of each biopsy location, 1 sample will be processed for histopathologic analysis, and the other sample for molecular analysis. Histopathologic analysis will be performed using hematoxylin-and-eosin staining and immunohistochemical markers to assess cellularity, glioma infiltration, proliferation,

microvascular changes, and necrosis. Molecular analysis will include assessment of DNA mutations, deletions, amplifications, and ribonucleic acid expression profiling. Two experienced neuropathologists blinded to the imaging results will evaluate all biopsies independently and will designate them as follows: normal brain tissue; diffuse glioma with few, moderate, or many tumor cells in a background of pre-existent brain tissue; highly cellular glioma without (apparent) pre-existent brain tissue remaining; uninformative.

DISCUSSION

Few studies investigate the diagnostic accuracy of glioma delineation, and most of these studies assess only 1 or 2 imaging modalities. This can at least partly be explained by the logistical challenge of multimodality preoperative imaging and of obtaining multiple image-guided biopsies. Nevertheless, studies that provide a direct comparison of multiple imaging modalities with histopathologic data are necessary to determine the optimal imaging modality for the delineation of diffuse gliomas. Using combined PET-MRI will help to reduce the number of scans necessary for multimodality imaging, while frameless stereotactic techniques will facilitate the acquisition of multiple image-guided biopsies with good accuracy within a limited time.

The importance of adequate glioma delineation is underscored by reports describing that (near) radiologically complete resection of MRI abnormalities (T1-weighted gadolinium-enhanced MRI for high-grade glioma and on T2 FLAIR-weighted MRI for low-grade glioma) is correlated with improved survival.[12-17] A resection based on modalities with superior delineation could result in even more complete resection and thus holds promise for even longer survival, and, conversely, for the identification of patients with glioma infiltration beyond meaningful surgical therapy, so that useless and possibly harmful resections can be avoided. Moreover, evidence accumulates that subsequent therapeutic modalities are more successful after resection that is as complete as possible.[16]

TRIAL STATUS

Patient recruitment was initiated on September 1, 2014.

SAFETY CONSIDERATIONS

Because neuronavigated biopsy has a risk of $<2\%$ of intracranial hemorrhage with consequences for the patient, the number of biopsy trajectories is limited to 3.[18,19] Because the biopsy procedure is immediately followed by a craniotomy for tumor resection, possible hemorrhages can be identified directly and removed. The tumor resection will be performed according to standard care.

All adverse events reported spontaneously by the subject or observed by the investigator or staff will be recorded in the protocol case report forms (CRF) using the Common Terminology Criteria for Adverse Events classification.[20] All serious adverse events will be reported through the web portal ToetsingOnline (https://www.toetsingonline.nl) to the accredited Medical Ethical Committee (METC) that approved the protocol. Serious adverse events that result in death or are life-threatening are reported expeditiously.

FOLLOW-UP

All patients will receive standard follow-up, which consists of postoperative clinical admission for as long as needed and an outpatient appointment 8 weeks after the procedure. Apart from that, postoperative adjuvant chemo- and/or radiotherapy will be installed according to histopathologic and molecular classification of the tumor, as discussed postoperatively at the neuro-oncology tumor board meetings. All adverse events will be followed until they have abated or until a stable situation has been reached.

DATA MANAGEMENT AND STATISTICAL ANALYSIS

Data will be collected on electronic CRF (eCRF). The eCRF is only assessable by the principal and the study investigator. The eCRF will be completed on-site by an investigator. The principal investigator will review the collected data.

The number of biopsies and patients required to compare the area under the curve (AUC) of the receiver operating characteristic (ROC) curves depend on the reference AUC (t1), the minimal relevant AUC from the improved imaging (t2), the ratio of nontumor and tumor biopsies (ratio), the correlation of imaging within patients (r), the average number of biopsies per patient (s), the correlation of histopathologic quantification between biopsies within patients (rho), the type I error (alpha), and the type II error (beta).[21-23] Under the assumptions of t1 0.6, t2 0.8, ratio 0.25, r 0.5, s 6, rho 0.2, alpha 0.05, and beta 0.2, 20 patients per glioma target volume subgroup are required. The overall study population then comprises 20 non-enhancing and 20 enhancing glioma patients, each stratum providing at least 120 biopsies. For testing the correlation between simplified and full quantitative measurement of input function in dynamic PET scanning, a sample size of 8 is mostly used in pilot studies. Due to experience with other trials, we will include this number in phase I. In phase II, 32 patients will be included to obtain the total of 40 patients from our sample size calculation.

Continuous variables will be described as a mean with standard deviation if the distribution is symmetric and as a median with minimum and maximum if it is skewed. Categoric variables are presented as numbers with percentages. Data analysis will be performed using R. AUCs are compared using a nonparametric resampling test using pROC in R.[24-26] Next, multivariate logistic regression analysis modeling

histopathology by quantitative imaging is performed using Bayesian models.

QUALITY ASSURANCE

As the METC of VU University Medical Center (VUmc) decided it was unnecessary to appoint a Data Safety Monitoring Board for this study, the progress of this study will be monitored by the Clinical Research Bureau of VUmc.

EXPECTED OUTCOMES OF THE STUDY

We expect that advanced imaging in combination with standard imaging will have a superior diagnostic accuracy for glioma delineation compared with current standard imaging. This delineation could help neurosurgeons, neurologists, radiation oncologists, and medical oncologists in their clinical decision-making. Next, studies comparing glioma resection or radiotherapy using standard vs standard plus advanced imaging can be conducted to investigate possible influences on clinical outcome.

The expected correlation between advanced imaging and histologic and molecular characteristics could provide biomarkers for prognosis and choice of therapy, as well as further insight into glioma imaging.

DURATION OF THE PROJECT

We anticipate that phase I will take 12 months and phase II 24 months, aiming for a total study duration of 3 years.

PROJECT MANAGEMENT

The principal investigator, Dr de Witt Hamer, will lead the study. Dr Pouwels will be responsible for the MRS data, Dr Barkhof for the MRI data, Dr Boellaard and Dr Hoekstra for the PET data, and Dr Wesseling for the pathology data. The study investigator, Mr Verburg, MSc, will coordinate the logistics of the study as well as the interpretation of the results.

ETHICS

The study is approved by the METC of VUmc and will be conducted according to the principles of the Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act. Explicit written consent will be obtained from all patients in this study.

Disclosures

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The investigation site is the VU University Medical Center (VUmc), P. O. Box 7057, 1007 MB Amsterdam, The Netherlands.

The principle investigator is P.C. de Witt Hamer, MD, PhD, Department of Neurosurgery.

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