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 (FLAIR) hyperintensity for non-enhancing 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 three 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.
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 to standard MRI alone. In addition, we anticipate that advanced imaging will correlate with the histopathologic and molecular characteristics of glioma.
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.

General information

The study is titled**:** 'Frontiers in advanced imaging of unexplored glioma regions (FRONTIER study)' (www.trialregister.nl, unique identifier NTR5354). Overall study dates are September 2014 to September 2017. Funding agencies: Cancer Center Amsterdam and the Dutch Cancer Society.

Investigation site: VU University Medical Center (VUmc), P.O. Box 7057, 1007 MB Amsterdam, The Netherlands.

Principle investigator: P.C. de Witt Hamer, MD, PhD, Department of Neurosurgery.

Investigators: W.P. Vandertop, MD, PhD, and N. Verburg, MD, Department of Neurosurgery; J.C. Reijneveld, MD, PhD, Department of Neurology; P. Wesseling, MD, PhD, Department of Pathology; P.J.W. Pouwels, PhD, Department of Physics & Medical Technology; F. Barkhof, MD, PhD, R. Boellaard, MSc, PhD and O.S. Hoekstra, MD, PhD, Department of Nuclear Medicine & PET Research.

Rationale and background information

Gliomas represent 80-90% 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] 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 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 non-enhancing 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 two centimeters beyond standard MRI outlines, underscores that up till 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 (DWI), perfusion-weighted imaging (PWI), 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, which so far has not been adequately answered: 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. Besides, we hypothesize that histological 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

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

Exclusion criteria

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

Withdrawal criteria

Patients who do not successfully undergo one PET scan. A summary of all criteria is given in table 1.

Study description

The study is separated into two phases (Figure 1). In both phases, standard and advanced imaging will be performed pre-operatively (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 are collected from each biopsy location, one for assessment of histopathologic characteristics and one 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 (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.

PET

PET will be performed using the Philips Gemini time-of-flight (TOF) PET-CT scanner or the Philips Ingenuity TOF PET/MRI-scanner. After intravenous administration of 370 megabecquerel (MBq) of $[^{15}O]H_2O$ a 10 min dynamic scan is acquired. This is followed by a 40 min dynamic scan after injection of 200 MBq CHO. With a minimum of 4 hours after injection of CHO the FET scan will be performed the same day using 200 MBq FET and a scan time of 90 minutes. During the scans manual blood samples are 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 one sample will be processed for histopathologic analysis and the other sample for molecular analysis. Histopathologic analysis will be performed using hematoxylin-and-eosin (H&E) 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 RNA expression profiling. Two experienced neuropathologists will evaluate independently, and blinded for the imaging results, all biopsies and designate those as: 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) preexistent brain tissue remaining; uninformative.

Discussion

Few studies investigate the diagnostic accuracy of glioma delineation, and most of these studies assess only one or two imaging modalities. This can at least partly be explained by the logistic 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 HGG and on T2/FLAIR-weighted MRI for LGG) 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 to identify 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 less than 2% of intracranial hemorrhage with consequences for the patient, the number of biopsy trajectories is limited to three. [18, 19] Since the biopsy procedure is immediately followed by a craniotomy for tumor resection, possible hemorrhages can be directly identified 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 his 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 (SAEs) will be reported through the web portal ToetsingOnline (https://www.toetsingonline.nl) to the accredited Medical Ethical Committee (METC) that approved the protocol. SAEs 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 eight 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 non-tumor 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 eight is mostly used in pilot studies. Due to the 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. Categorical 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 versus 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 three 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 and 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

Financial support was provided by grant CCA2012-2-05 of the Cancer Center Amsterdam (CCA) of the VU University Medical Center and grant OAA/H1/VU 2015-7502 of the Dutch Cancer Society.

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Figures and Tables

Table. 1 Inclusion/Exclusion/Withdrawal criteria. MRI = magnetic resonance imaging, PET = positron emission tomography

Fig. 1 Imaging protocol for different phases study. Cho = 11C-Choline, FET = [18F]Fluoroethyl-tyrosine

Table.2 Quantitative imaging parameters. MRI = magnetic resonance imaging, PET = positron emission tomography, FLAIR = Fluid attenuated inversion recovery, T/N ratio = tumor-to-normal radioactivity (PET) or signal intensity (MRI), MRS = Magnetic Resonance Spectroscopy, Cho = choline, NAA = N-acetyl aspartate, ASL = Arterial Spin Labeling, CBF = Cerebral Blood Flow, DTI = Diffusion Tensor Imaging, FA = Fractional Anisotropy, ADC = Apparent Diffusion Coefficient, DSC = Dynamic Susceptibility Contrast, CBV = Cerebral Blood Volume, SUV = Standardized uptake value