Clinical trials registration number: NCT02186262

Status: Patient enrolment ongoing

1 SUMMARY

Grading of gliomas is of significant clinical importance since the prognosis as well as the treatment of choice are distinct in low-grade and high-grade gliomas. With standard MRI modalities, however, a reliable distinction is often impossible. Moreover, the gold standard for glioma grading by histopathology may also have limitations due to unrepresentative tumor samples. The advantage of MRI is the ability to 'sample' the entire lesion. Therefore, more advanced MRI techniques are urgently needed that would have higher sensitivity and specificity in the definition of tumor type, grade and extent. The aim of our study is to develop novel imaging protocols suitable for the MRI of glioma using advanced MRI techniques such as RAFF relaxation and DWI modeling. In addition, we want to study the correlation between advanced MRI parameters and histopathology of the tumor specimen.

2 INTRODUCTION

Gliomas are the most common primary brain neoplasms. They range from low grade to anaplastic and glioblastoma multiforme (GBM) which are also referred to as high grade gliomas. The current standard criterion for tumor grading is based on the histopathologic assessment of tumor specimen obtained during surgery. However, this may have severe limitations due to sampling errors especially when tumor-grade heterogeneity is presented within the same tumor specimen. These sampling errors could have profound consequences on the management of the disease and survival. Therefore, novel more accurate non-invasive imaging tools are needed that could predict cancer aggressiveness and thus complement the histopathologic grade. (1).

It is well known that radiotherapy may induce necrosis that manifests as oedema and enhancement visible on dynamic contrast enhanced magnetic resonance imaging (DCE-MRI). This radiation necrosis is usually impossible to distinguish from tumor progression using just conventional anatomical MRI (2). In addition, a transient increase in apparent tumor size and enhancement after therapy are increasingly being observed in patients with glioblastoma since chemoradiotherapy with temozolomide was established as the new standard of care (3).

Nevertheless, it is of utmost clinical importance to define patients who are responding to therapy from those who require change in the therapy plan. Therefore, more advanced non-invasive imaging methods monitoring the response to treatment are urgently needed.

3.2. Anatomical magnetic resonance imaging

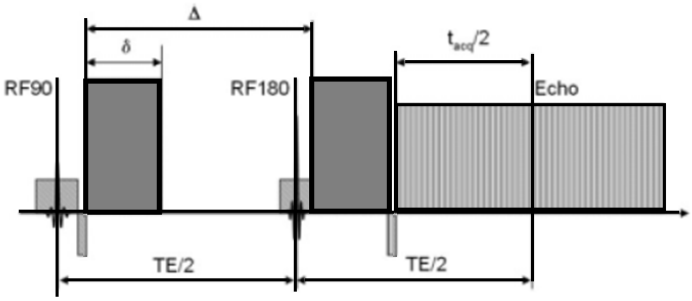
Gliomas tend to have high signal on T2 based sequences. The hyperintensity on T2 based sequences (including fluid-attenuated inversion-recovery, FLAIR) is due to prolongation of transverse relaxation times (T_2 relaxation time) mainly caused by the increase in tissue water content and ultrastructural changes. The areas of haemosiderin appear as foci of signal dropout.

3.2. Rotating frame imaging along fiction filed (RAFF) and $T1_\rho$ imaging

Relaxation along a fictitious field (RAFF) is an MR imaging technique applying amplitude and frequency-modulated irradiation in a subadiabatic regime. The use of radiofrequencypulse is based on sine and cosine amplitude and frequency modulations of equal amplitudes, which give rise to a stationary fictitious magnetic field in a doubly rotating frame. The RAFF relaxation time constant (T_{RAFF}) was found to differ from laboratory frame relaxation times (T_1 and T_2) and rotating frame relaxation times ($T_{1\rho}$ and $T_{2\rho}$) (4). Rotating frame relaxations ($T_{1\rho}$ and T_{RAFF}) have shown to be quantitative MRI markers to follow up disease progression, including brain and myocardial ischemia (5) and to follow up response to therapy(6). Moreover, T_{RAFF} has shown excellent correlation with cell density in a rat glioma model, which makes it a potential biomarker to follow up cancer therapy outcome (7).

3.2. Diffusion weighted imaging (DWI) and diffusion tensor imaging (DTI)

In order to measure diffusion properties of tissue using MRI, the Stejskal–Tanner imaging sequence is used (Stejskal and Tanner, 1965). Information about proton diffusion is acquired by applying motion-encoding gradients, which cause phase shifts in moving protons. This phase shift depends on the quantity as well as direction of movement.



where γ denotes the gyromagnetic ratio, G the diffusion gradient amplitude, δ the diffusion gradient duration and Δ the time between the leading edges of the diffusion gradient pulses.

It was demonstrated that fractional anisotropy (FA) and apparent diffusion coefficient (ADC) correlate with glioma grade (8). In addition, DTI could reveal larger peritumoral abnormalities in gliomas, which are not apparent on conventional anatomical MRI (9).

3.4. Proton magnetic resonance spectroscopy (^{1}H MRS)

Proton magnetic resonance spectroscopy (^{1}H MRS) has the ability to detect metabolite concentrations in brain tissue. It allows evaluation of brain metabolites such as N-acetylaspartate (NAA), total choline (Cho), and total creatine (Cre). In certain conditions additional signals including lactate, in the presence of anaerobic metabolism, and lipids, in areas of cellular breakdown caused by necrosis, are detected. Commonly, elevated Cho, decreased NAA, and the presence of lipids and lactate are typical findings in malignant gliomas. Ratios of these metabolities were also shown to be useful in tumor grading (10).

3.6. Study hypothesis

Our hypothesis is that improved preoperative staging, characterization and target delineation of brain tumors using novel MRI techniques, including RAFF relaxation and advanced DWI modeling, provides tools for better treatment selection and non-invasive detection of cancer aggressiveness. In addition, we hypothesize that advanced MRI could enable accurate monitoring of therapy response.

3 OBJECTIVES AND PURPOSE

Specific aims of this project are as follows:

i) To measure quantitative MR relaxation values ((T_{RAFF}, T_1, T_2, $T_{1\rho}$, $T_{2\rho}$) of human gliomas

ii) To develop quantitative and qualitative methods for evaluation of advanced MRI techniques

iii) To develop and validate a novel imaging protocol suitable for MRI of glioma

iv) To study the correlation between advanced MRI parameters (T_{RAFF},$T_{1\rho}$, ADC, FA, Cho, Cr, NAA) and histopathology as well as the molecular markers of the tumor specimen

4 STUDY DESIGN

This is an open prospective study to obtain information on applicability of RAFF and DWI methods for the purpose of therapy planning and detection of glioma aggressiveness. The use of RAFF and DWI could provide useful information about tumor microenvironment. It is hypothesized that RAFF and DWI show non-invasively heterogeneous distribution ingliomas. Combination of RAFF with other MR imaging techniques and ^{1}H MRS could provide non-invasive tools for detection of glioma aggressiveness. If the hypothesis is proven both advanced MRI techniques may be implemented to therapy planning as well as monitoring of therapy response.

5 PATIENT SELECTION

5.1 Source population

All new cases of gliomas are referred for neurosurcigal treatment to Turku University Hospital from the Hospital Districts of Southwestern Finland, Satakunta, and Åland. Recurrent gliomas are also referred to the Department of Neurosurgery to evaluate possibility of re-operation. The total population in these three hospital districts is approximately 724000. Number of eligible patients in one year is 30 – 50.

5.2 Number of patients

This study comprises of two groups: primary (Group I) and recurrent malignant gliomas (Group II) who are scheduled for an operation. Both series will include a maximum of 40 patients.

5.3 Inclusion criteria

- Age: 18 to 80 years old
- Language spoken: Finnish or Swedish
- Performance status: Karnofsky score 70 or better or WHO performance status 2 or better
- Supratentorial primary malignant glioma (the diagnosis is based on radiological and clinical grounds)
- Supratentorial recurrent glioma based on MRI and/or [^{11}C]methionine PET imaging
- Patient is scheduled to either surgery or stereotactic biopsy
- Mental status: Patients must be able to understand the meaning of the study
- Informed consent: The patient must sign the appropriate Ethical Committee (EC) approved informed consent documents in the presence of the designated staff

5.4 Exclusion criteria

- Prior medical history: Patient must have no history of serious cardiovascular, liver or kidney disease
- Any psychiatric condition that compromises the subject's ability to participate in the study
- Infections: Patient must not have an uncontrolled serious infection
- No contraindications for MRI (cardiac pacemaker, intracranial clips etc)
- Patient must not have claustrophobia with serious symptoms
- Pregnant or lactating women

6 MULTIMODALITY IMAGING

6.1 Pre-study evaluation

All patients are first evaluated by a neurosurgeon. Patients meeting inclusion criteria but not any exclusion criteria will be asked to participate in the study. Patients will be informed orally and in written form about the study and if they are willing to participate in it they will be

asked to sign the informed consent form. Typically they will be allowed 2-4 days to read the patient inform sheet preferably with their close relatives before their consent to participate is requested.

7.2 MRI

MRI imaging of the brain is done over 40 minutes. Possible imaging devices are1.5T or 3T Siemens system (Magnetom Aera 1.5T or Verio 3T, Erlangen, Germany) or 1.5T/3T Philips system (Philips Ingenia, Best, Netherlands) or 3T Philips PET/MR system (Philips Ingenuity, Best, Netherlands). Integrated RF coil will be used for excitation while dedicated 32 channel coil will be used for signal perception. Initially, data for attenuation correction will be obtained followed by anatomical sequences such as T_2-weighted turbo spin echo, FLAIR and T_1-weighted anatomic imaging in axial, sagittal and coronal directions followed by RAFF and continues wave $T_{1\rho}$. The obtained images will be used for planning of 2D ^{1}H MRS examination, based on PRESS sequences, of selected brain area. Diffusion weighted imaging will be obtained with single-shot 2D spin-echo echo-planar imaging. No gadolinium-enhanced T1-weighted imaging will be performed.

7.3 Clinical follow up

After MRI scanning the patient can return home or the hospital unit depending on what had been agreed with the neurosurgeon. Treatment and follow-up ensues according to standard procedures in the departments of neurosurgery or oncology and radiotherapy. Tumor biopsies will be obtained during the surgical resection of the tumor and studied for histopathology as well as for molecular markers.

7 ADVERSE EVENTS

The risks for the patients inflicted by participation in the study are deemed minimal. Anatomical MRI and advanced MRI techniques are considered as safe techniques since no ionizing irradiation is used. In addition, no intravenous catheters are required since no paramagnetic contrast agents will be used. The presence of claustrophobia will be evaluated in the screening phase and patients with serious symptoms will be excluded from study.

8 ETHICS

8.1 Ethical considerations

The study will be conducted in compliance with the current revision of Declaration of Helsinki guiding physicians and medical research involving human subjects (59nd World Medical Association General Assembly, Soul, Korea, 2008).

8.2 Ethical Review

Prior to commencement of this investigation, the study protocol, patient information sheet and informed consent form will be submitted for approval to EC of the Hospital District of Southwest Finland. The Principal Investigator (PI) is responsible for obtaining approval of the EC for the study protocol including its appendices. The PI shall file all correspondence with the EC in the Investigator`s Study File.

8.3 Potential risks and benefits to study subjects

The risks for the patient inflicted by participation in the study are deemed minimal. Both standard MRI and advanced MRI are considered as safe techniques. Though participating in the study does not provide direct medical benefit for the patient it may contribute to the development of higher quality MRI imaging in brain tumors.

9 DATA ANALYSIS

9.1 Quantitative analysis of DWI data

Several b-values will be used in calculations.

10.4.1.Monoexponential quantification of DWI

Monoexponential calculation of apparent diffusion coefficient (ADC) can be described by following equation (eq.1):

$$ADC = -\ln\left(\frac{SI(b_2)}{SI(b_1)}\right) \qquad \text{(eq. 1)}$$

where SI(b_2) and SI(b_1) denotes the signal intensity at higher (b_2) and lower b-value (b_1).

10.4.2. Biexponential quantification of DWI using high b-values

In that case the following biexponential function (eq. 2) can be used to describe signal decay:

$$\frac{S(b)}{S(0)} = (1-f)\cdot\exp(-b\cdot Ds) + f\cdot\exp(-b\cdot Df), \qquad \text{(eq. 2)}$$

where *f* is the fraction of fast diffusion, *Ds* represents the slow components of diffusion and *Df* represents the fast components of diffusion.

10.4.3. Biexponential quantification of DWI using low b-values

The intravoxel incoherent motion (IVIM) theory is an advanced method to separate diffusion and perfusion effects using DWI (11) at low b-values. The IVIM theory states that the blood flow in the capillaries causes a dephasing of the blood magnetization when motion-encoding gradients are applied. This means that the motion of water molecules due to microcirculation of blood in the capillary network (perfusion) has a similar impact on of resulting MRI signal as their motion due to molecular diffusion.

IVIM can be expressed by the following biexponential equation (eq. 3):

$$\frac{S(b)}{S(0)} = f\cdot\exp(-b\cdot D^*) + (1-f)\cdot\exp(-b\cdot D), \qquad \text{(eq. 3)}$$

where *f* is the perfusion fraction, D^* is the perfusion components of diffusion and *D* represents the fast components of diffusion.

9.2 Quantitative analysis of ^{1}H MRS data

Different metabolic ratios will be measured. Quantification of the metabolite-to-citrate peak area ratio was accomplished by LCModel (version 6.2-1L). LCModel spectrum processing does not use apodization of the time domain signals to improve SNR. The algorithm fits spectra using simulated basis functions created for each metabolite. Cramer-Rao minimal lower band (%SD) is therefore underestimated. This is typical for empirical peak-fitting methods. SNR < 3

(vendor's recommendation) will be used as a criterion to reject spectra instead of %SD. The following spectral lines will be fitted N-acetylaspartate (NAA), total choline (Cho), and total creatine (Cre) lactate (Lac), lipids and their LCModel fits, phase corrected spectra and baseline will be normalized by summing the squares of the intensities of the spectral points in each spectrum and then dividing the amplitude of each point by the square root of this sum. Mean normalized spectra will be calculated by averaging the values at each data point.

9.3 Quantitative analysis of RAFF data

Mono-exponential function will used to fit signal decay of the RAFF measurements.

9.4 Statistical analysis

All analyses will be performed with SAS version 9.1 (SAS Institute, Inc., Cary, NC). A p-value of <0.05 will be considered to be statistically significant.

10 SAMPLE SIZE

This prospective feasibility study which assesses utility of advanced MRI techniques for detection and characterization of brain tumors will enroll 80 patients. An interim analysis will be made after 15 patients with emphasis on imaging characteristics and the study may be interrupted at the discretion of principal investigator after consulting other chief investigators.

11 QUALITY ASSURANCE

11.1 Training and information of study personnel

The technical and other supporting personnel of Turku PET Centre and Department of Diagnostic Radiology are well experienced in performing MRI studies. In the beginning of the study all involved personnel will be informed on the practical implementation of the protocol in a separate institutional meeting. They will be informed on the rationale of the study and possible clinical implications as well.

11.2 Protocol amendments

According to Finnish national regulations, protocol amendments can be made if all investigators agree. They are presented in a written form and dated as applicable. They include the original chapter of the study protocol and the amended chapter, with an explanation to this change. Important protocol amendments are reviewed by the local Ethical Committee.

12 STUDY SCHEDULE

The study will start in January 2014 pending all mandatory authorizations have been obtained. All MRI studies are expected to be performed within two years. Analysis and modeling of the MRI data is feasible once 7-10 patients have been imaged. Preliminary analysis of results will be available in late 2014 and first reports are expected to be written during 2014-2015.

13 FINANCING

The study will be financed in part by Finnish Governmental Special Funding (In Finnish: ’Erityisvaltionosuus, EVO’). Additional funding is sought through national non-profit organizations such as Sigrid Juselius Foundation and Cancer Foundations of Finland.

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