# Brain Tumor Imaging and Treatment Effects. Imaging findings and cognitive function in glioblastoma patients.

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# Brain Tumor Imaging and Treatment Effects

## Imaging findings and cognitive function in glioblastoma patients

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# Brain Tumor Imaging and Treatment Effects

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Imaging findings and cognitive function in glioblastoma patients

**Anna Rydelius**

LUND
UNIVERSITY

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**Title and subtitle** Brain tumor imaging and treament effects
Imaging findings and cognitive function in glioblastoma patients


**Abstract**

**Background:** Glioblastoma is the most common malignant brain tumor. Operation with maximal resection, if feasible, otherwise biopsy followed by radiotherapy and chemotherapy with temozolomide is standard therapy. The prognosis remains poor, with median overall survival being 15 months despite therapy. Improved monitoring and treatment response assessment will be important when seeking to improve treatment efficacy and patient quality of life.

**Aims:** The present work sought to follow newly diagnosed glioblastoma patients by imaging and clinical monitoring. Specific aims were to study the impact of surgical resection degree on prognosis and the effects of currently used therapies, including arc-based rotation radiotherapy, longitudinally. Aims were also to study radiological parameters with advanced magnetic resonance imaging (MRI) as well as patient neurological and cognitive functions in order to early identify prognostic factors.

**Material and methods:** In paper I, volumetric assessment by quantitative and subjective methods was retrospectively studied from pre- and postoperative MRI in glioblastoma patients undergoing tumor resection. Influence of extent of resection of contrast enhanced tumor on progression-free survival and overall survival was analyzed, measured as relative extent of resection (EOR) and absolute residual tumor volume (RTV).
In the present MRI brain tumor study, patients newly diagnosed with glioblastoma undergoing treatment with arc-based radiotherapy were studied longitudinally over a one-year period and constituted the patient cohort of papers II-IV, using advanced MRI, including diffusion-weighted imaging sequences. Microstructural changes in non-tumorous brain structures, including white matter (corpus callosum, centrum semiovale) and the limbic system (hippocampus, amygdala), were assessed by diffusion tensor imaging (DTI) during and after irradiation. By parametric response mapping (PRM), changes of mean diffusivity (MD) in tumor regions were analyzed as MD-PRM. Baseline examinations were compared with examinations 3 weeks into radiotherapy voxel-wise, analyzing the MD-difference as prediction of therapy response and survival. Clinical parameters were monitored from start of radiotherapy up to one year and included correlation of cognition, measured by the computerized test-battery CNS-vital signs (CNS-VS), with therapy and disease progression.

**Results:** Quantitative volumetric measurements, especially residual tumor volume of ≤1,6 mL, showed prognostic significance for longer progression-free and overall survival. The quantitative volumetric method was superior in reproducibility compared to conventional estimation. MD-PRM demonstrated that in patients only undergoing diagnostic biopsy MD-PRM changes indicated prognostic specificity for treatment response at 8 months. Significant longitudinal DTI changes were only observed in the body of the corpus callosum during and up to one year from radiotherapy. Evaluation of cognitive performance in glioblastoma patients using cognitive test scores by CNS-VS at baseline were in lower-average or low, compared to standard test average in 4 main domains: executive function, visual and verbal memory and complex attention. Cognitive function remained stable without further deterioration during one year follow up after radiotherapy was initiated. Better cognitive function at therapy begin correlated with longer progression-free and overall survival.

**Conclusion:** Quantitative volumetric assessment has prognostic impact on glioblastoma patients progression-free and overall survival in favor of gross total resection. MD-PRM could not predict treatment response as assessed in the entire patient cohort, but may have predictive value in biopsied patients. Longitudinal monitoring up to one year after initiated radiotherapy did not reveal any major changes, neither in microstructural changes by DTI parameters, nor in patients cognitive function, indicating less neurotoxicity by arc-based radiotherapy.



**Key words** Glioblastoma, volumetry, congitive function, DTI, MD-PRM, arc-based radiotherapy

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# Abstract

*Background:* Glioblastoma is the most common malignant brain tumor. Operation with maximal resection, if feasible, otherwise biopsy followed by radiotherapy and chemotherapy with temozolomide is standard therapy. The prognosis remains poor, with median overall survival being 15 months despite therapy. Improved monitoring and treatment response assessment will be important when seeking to improve treatment efficacy and patient quality of life.

*Aims:* The present work sought to follow newly diagnosed glioblastoma patients by imaging and clinical monitoring. Specific aims were to study the impact of surgical resection degree on prognosis and the effects of currently used therapies, including arc-based rotation radiotherapy, longitudinally. Aims were also to study radiological parameters with advanced magnetic resonance imaging (MRI) as well as patient neurological and cognitive functions in order to early identify prognostic factors.

*Material and methods:* In paper I, volumetric assessment by quantitative and subjective methods was retrospectively studied from pre- and postoperative MRI in glioblastoma patients undergoing tumor resection. Influence of extent of resection of contrast enhanced tumor on progression-free survival and overall survival was analyzed, measured as relative extent of resection (EOR) and absolute residual tumor volume (RTV).

In the present MRI brain tumor study, patients newly diagnosed with glioblastoma undergoing treatment with arc-based radiotherapy were studied longitudinally over a one-year period and constituted the patient cohort of papers II-IV, using advanced MRI, including diffusion-weighted imaging sequences. Microstructural changes in non-tumorous brain structures, including white matter (corpus callosum, centrum semiovale) and the limbic system (hippocampus, amygdala), were assessed by diffusion tensor imaging (DTI) during and after irradiation. By parametric response mapping (PRM) changes of mean diffusivity (MD) in tumor regions were analyzed as MD-PRM. Baseline examinations were compared with examinations 3 weeks into radiotherapy voxel-wise, analyzing the MD-difference as prediction of therapy response and survival. Clinical parameters were monitored from start of radiotherapy up to one year and included correlation of cognition, measured by the computerized test-battery CNS-vital signs (CNS-VS), with therapy and disease progression.

*Results:* Quantitative volumetric measurements, especially residual tumor volume of ≤1,6 mL, showed prognostic significance for longer progression-free and overall survival. The quantitative volumetric method was superior in reproducibility compared to conventional estimation. MD-PRM demonstrated that in patients only undergoing diagnostic biopsy MD-PRM, changes indicated prognostic specificity for treatment response at 8 months. Significant longitudinal DTI changes were only observed in the body of the corpus callosum during and up to one year from radiotherapy. Evaluation of cognitive performance in glioblastoma patients using cognitive test scores by CNS-VS at baseline were in lower-average or low, compared to standard test average in 4 main domains: executive function, visual and verbal memory and complex attention. Cognitive function remained stable without further deterioration during one year follow up after radiotherapy was initiated. Better cognitive function at therapy begin correlated with longer progression-free and overall survival.

*Conclusion:* Quantitative volumetric assessment has prognostic impact on glioblastoma patients progression-free and overall survival in favor of gross total resection. MD-PRM could not predict treatment response as assessed in the entire patient cohort, but may have predictive value in biopsied patients. Longitudinal monitoring up to one year after initiated radiotherapy did not reveal any major changes, neither in microstructural changes by diffusion tensor imaging (DTI) parameters, nor in patients cognitive function, indicating less neurotoxicity by arc-based radiotherapy.

# Populärvetenskaplig sammanfattning

## Hjärntumör inklusive glioblastom

Glioblastom är den vanligaste sortens hjärntumör bland vuxna och samtidigt den mest elakartade. Glioblastom drabbar cirka 550 personer årligen i Sverige. När tumören upptäcks är behandlingen först operation för exakt diagnos och med syfte att avlägsna så mycket som det går av tumören, utan att orsaka skada för patienten, annars görs en diagnostisk biopsi. Standardbehandling därefter är strålbehandling med fotoner och samtidig (s.k. konkomitant) cellgiftsbehandling med Temozolomid. Även med standardbehandling är prognosen allvarlig med genomsnittlig överlevnad på ca 15 månader. Trots att mycket intensiv forskning pågår sedan årtionden för att hitta bot, har det hittills endast resulterat i begränsad framgång.

Det är viktigt att hantera symtom som tumörsjukdomen ger upphov till med stöd och symptomlindrande behandling. Vanligaste symptom är olika former av neurologiska bortfall såsom tal- och synpåverkan, kraftnedsättning, känselstörning, minnes- och koncentrationspåverkan. Samtidigt med symptomlindring behöver tumörbehandlingens effekt och eventuella biverkningar övervakas och följas upp. Detta för att säkerställa bästa behandlingssvar och för att uppnå så god livskvalitet som möjligt.

## Fokus och målsättning

I detta avhandlingsarbete har vi haft som avsikt att följa glioblastom-patienter med bildgivande radiologisk diagnostik och klinisk uppföljning. Först genom att undersöka betydelsen av att exakt mäta hur mycket tumör som kunnat opereras bort och hur det påverkar prognosen för patienten. Vidare genom att undersöka över tid hur nuvarande behandlingsmetoder inklusive uppdaterad fotonstrålbehandling med arc-based (båg-baserad) rotations strålning påverkar individen. Det studerades parallellt dels med bildgivande metoder med avancerade magnet resonanstomografi (MR), dels genom fortlöpande neurologisk och kognitiv undersökning och livskvalitetsenkäter för att tidigt kunna identifiera faktorer som påverkar patienten och prognosen.

# Delarbeten

I det första arbetet (I) undersöktes genomförda MR undersökningar från patienter opererade för glioblastom före och efter operation med både visuell som exakt volumetrisk mätmetod där man mätte procent bortopererad tumör och volym i milliliter kvarvarande tumör.

I MR studien som innefattar delarbeten II-IV inkluderas nydiagnostiserade glioblastompatienter som sedan följdes upp regelbundet med avancerad MR och kliniska och kognitiva undersökningar under och efter strålbehandling. I det kognitva delarbetet (II) testades patienternas kognitiva förmåga med det datorbaserade testet CNS-vital signs (CNS-VS). I arbetet om parametriska responskartor (PRM, delarbete IV) mättes medel-diffusionen i tumörvävnad från MR undersökning vid strålstart och efter 3 veckor för att se om man med denna metod tidigt kan förutsäga behandlingseffekt. I delarbetet om strålreaktion i bestrålad hjärnvävnad (III) mättes s.k. diffusion tensor imaging (DTI) som är en MR metod för att studera vävnadens mikrostruktur.

# Resultat och konklusioner

Volymsmätning av mängden bortopererad tumör visade att det var längst tid av stabilt tillstånd och längst överlevnad för de patienter där man kunnat operera bort maximalt stor andel av tumören med minimal eller ingen kvarvarande tumör.

Vid kognitiv testning vid behandlingsstart hade glioblastom-patienterna i genomsnitt en något nedsatt kognitiv förmåga jämfört med standardresultat från friska individer. De mest betydelsefulla kognitiva symptom var nedsatt uppmärksamhet, exekutiv förmåga och nedsatt språk- och bildminne. Den kognitiva förmågan ändrades inte ytterligare efter strålbehandling. De individer som hade god kognitiv funktion vid behandlingsstart hade längre tid av stabilt tillstånd och överlevnad. Tidig kognitiv testning kan således visa tecken på förväntat behandlingssvar och kan vara värdefull för glioblastom-patientens fortsatta vård och planering.

Undersökning av medel-diffusions PRM (MD-PRM) kunde inte påvisa någon tidig skillnad för att förutsäga behandlingssvar i hela gruppen. Däremot kunde vi se tecken hos den del av patienterna som endast blivit opererade med biopsi, att kunna förutsäga behandlingssvar vid 3 veckor in i behandlingen genom MD-PRM resultaten. MD-PRM kan därför tänkas som stöd i planeringen för glioblastom-patienter som endast kunnat genomgå biopsi.

Analys av mikrostruktur i bestrålad hjärnvävnad med MR metoden diffusion tensor imaging (DTI) visade endast mycket begränsad förändring av DTI i hjärnvävnadens

mikrostruktur under och efter strålbehandling. Dessa fynd skiljer sig från tidigare DTI studier som visat större förändring under och efter strålbehandling.

Vi tolkar dessa resultat som att modern strålbehandlingsteknik med arc-based terapi inte påverkar hjärnan så negativt som tidigare beskrivits, varken gällande kognitiv förmåga eller hjärnans mikrostruktur. Således tolkar vi att den nyare strålbehandlingsmetoden är mer skonsam för patienten än tidigare metoder.

# Original papers

I. Adam Blomstergren,* Anna Rydelius,* Kasim Abul-Kasim, Jimmy Lätt, Pia C. Sundgren and Johan Bengzon. Evaluation of reproducibility in MRI quantitative volumetric assessment and its role in the prediction of overall survival and progression-free survival in glioblastoma. Acta Radiol. 2019 Apr;60(4):516-525. doi: 10.1177/0284185118786060.

II. Anna Rydelius , Jimmy Lätt, Sara Kinhult, Silke Engelholm, Danielle Van Westen, Mats Pihlsgård, Johan Bengzon, Pia C. Sundgren** and Åsa Lilja**. Longitudinal study of cognitive function in glioma patients treated with modern radiotherapy techniques and standard chemotherapy. Acta Oncol. 2020 Sep;59(9):1091-1097. doi: 10.1080/0284186X.2020.1778181.

III. Anna Rydelius, Björn Lampinen, Andreas Rundcrantz, Johan Bengzon, Silke Engelholm, Danielle vanWesten, Sara Kinhult, Linda Knutsson, Jimmy Lätt, Markus Nilsson and Pia C. Sundgren. Diffusion tensor imaging in glioblastoma patients treated with volumetric modulated arc radiotherapy: a longitudinal study. Acta Oncol. 2022 Jun;61(6):680-687. doi: 10.1080/0284186X.2022.2045036.

*IV.* Rydelius A, Bengzon J, Engelholm S, Kinhult S, Englund E, Nilsson M, Lätt J, Lampinen B** Sundgren PC**. Predictive value of diffusion MRI-based parametric response mapping for prognosis in glioblastoma. Manuscript in submission to MRI. *Manuscript submitted to Magnetic Resonance Imaging.*

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* Shared first authors due to equal contribution.

** Shared last authors due to equal contribution.

# Abbreviations

| | |
|---|---|
| 3D-CRT | 3-dimentional Conformal RadioTherapy |
| 5-ALA | 5-aminolevulinic acid |
| ATRX | Alpha Thalassemia/mental Retardation syndrome X-linked gene |
| CNS | Central Nervous System |
| CNS-VS | Central Nervous System -Vital Signs |
| CT | Computer Tomography |
| DSC | Dynamic Susceptibility Contrast (perfusion MR) |
| DTI | Diffusion Tensor Imaging |
| DWI | Diffusion-Weighted Imaging |
| ECOG | Eastern Cooperative Oncology Group |
| EGFR | Epidermal Growth Factor Receptor |
| EOR | Extent of Resection |
| FLAIR | Fluid Attenuated Inversion Recovery |
| fMRI | functional MRI |
| HT | Helical Tomotherapy |
| IDH1/2 | Isocitrate Dehydrogenase 1 and 2 IDH 1/2 |
| IMRT | Intensity Modulated RadioTherapy |
| MGMT O(6) | Methylguanine-DNA Methyltransferase |
| MPRAGE | Magnetization Prepared Rapid Acquisition Gradient Echo |
| MRI | Magnetic Resonance Imaging |
| MRS | Magnetic Resonance Spectroscopy |
| OAR | Organs At Risk |
| pMRI | perfusion MRI |

| | |
|---|---|
| RANO | Responce Assessment in Neuro-Oncology |
| rCBV | relative Cerebral Blood Volume |
| RT | RadioTherapy |
| RTV | Residual Tumor Volume |
| T1 | T1-weighted sequences |
| T2 | T2-weighted sequences |
| TERT | Telomerase Reverse Transcriptase |
| TMZ | Temozolomide |
| TTFields | tumor treating fields |
| VMAT | Volumetric Modulated Arc Therapy |
| WHO | World Health Organisation |
| wt | Wide type, non-mutated |

# Introduction

Malignant brain tumors such as glioblastoma may affect healthy persons in the middle of their lives, often without prior medical history. The prognosis is severe. Despite improvement in surgical and oncological treatment methods and techniques, these are rarely curative, but limited to slowing disease progression and therefore they need to be combined with symptomatic therapy and supportive care in order to optimize patient function in everyday living and their quality of life. Understanding patients cognitive function at an early stage of the disease is valuable for further planning. Advanced imaging may aid improved individual treatment planning and follow up of glioblastoma patients. With this aim, advanced imaging could have positive impact on both survival and quality of life.

## Aetiology and epidemiology of gliomas

Glioma constitutes the most common type of primary malignant brain tumor (approximately 80%) but can occasionally also occur elsewhere in the central nervous system (CNS). Gliomas are considered to derive from the glial cells of the CNS. Glial cells are divided into astrocytes, oligodendrocytes, microglial cells and ependymal cells and each subpopulation has multiple interactive functions with neurons. The tumor subgroup of astrocytomas has been thought to origin from astrocytic cells whereas oligodendrogliomas are considered to have their origin in the myelin producing oligodendrocytes. Gliomas are traditionally graded from I to IV, where grade IV is highly aggressive. Glioblastoma WHO (World Health Organisation) grade IV, (GBM) is the most malignant, and also the most frequently occurring type of glioma. GBM is to this date marred by a short median survival, with only limited effect of therapy.

In adults, gliomas are most commonly located in the cerebral hemispheres, whereas in childhood the most common location is in the posterior fossa.

Worldwide the incidence rate of glioma varies from 4.7 to 5.7/100 000 persons [1]. Specifically glioblastoma WHO grade IV had an incidence of 0.59 to 3.69 per 100 000 persons worldwide in 2014. [1]

In Sweden, approximately 1400 patients (in a population of 10 000 000) are diagnosed with primary CNS tumors/year and of these 40% are glioma. https://sdb.socialstyrelsen.se/if_can/val.aspx.

In 2019; 521 new malignant glioma cases were diagnosed in Sweden, with an incidence rate of 6.4/100 000; 7.18/100 000 in male and 4,97/100 000 in female patients which include both anaplastic astrocytoma grade III and GBM grade IV.

In Denmark the incidence of glioma was 7.3/100 000 new cases/year, of which 5.1/100 000/year were glioblastoma, observed during 2009-2014 [2].

Notably, there are indications of a higher incidence of gliomas in Scandinavian countries than both the global average and comparable high-income economies such as Japan [1].

Gliomas vary in distribution depending on type of tumor and age of the patient. High grade glioma affect males more frequently than females with a 1.5:1 male to female ratio [1,3]. The risk for glioblastoma grade IV increases from the age of 60, peaking at an age of 75-84, whereas lower grade gliomas such, as diffuse astrocytoma and oligodendroglioma WHO grade II, are more commonly diagnosed in younger adults from the age of 30 [1,3].

The vast majority of gliomas occur without known underlying risk factors. However, previous exposure to high-dose ionizing radiation of the brain, such as administered to children with leukemia, or to children with juvenile brain tumors, are known risk factors for glioma. Interestingly, the risk is lower among individuals with allergies and some other auto-immune conditions compared to non-allergic individuals [1,4]. Rare hereditary risk factors, including syndromes such as neurofibromatosis type 1 (astrocytoma, optic nerve glioma), tuberous sclerosis (subependymal giant cell astrocytoma), Lynch Syndrome and Li–Fraumeni syndrome (glioblastoma, other gliomas), and Ollier disease/Maffucci syndrome (glioma) are associated with increased the risks of CNS tumor development. It is commonly assumed that specific families with more than two family members affected, have higher risks of glioblastoma. Although extensive studies are ongoing, especially the multicenter Case-Control Study by the Genetic Epidemiology of Glioma International Consortium studying cases of familial glioma [5,6], to the best of my knowledge, no further data on other specific hereditary risk genes, has been published to this date.

# Diagnostic classification, pathology and genetics

An initial preliminary clinical diagnosis is based on specific tumor associated radiological findings, as often revealed by initial computer tomography (CT) and by confirming semi-acute magnetic resonance imaging (MRI), further described below

in section Radiological features and primary diagnostics. Following tumor resection or biopsy, a final verified histopathological diagnosis is obtained. At a subsequent multi-disciplinary conference, attended by the neurooncologist, neurologist, neurosurgeon, neuroradiologist, neuropathologist and neuro-oncology nurse, follow-up and postoperative treatment plan is suggested.

## The World Health Organization (WHO) Classification

For exact histochemical diagnosis of glioma, the WHO classification of brain tumors, later called tumors of the nervous system, is used. The first version was published in 1993 [7] and has been under gradual development, as the knowledge of molecular genetics has improved along with and an increasing use of molecular profiling has shown significant impact on prognosis and possibly on aethiology. The WHO classification of CNS tumors increasingly influences treatment recommendations.

Gliomas in adulthood (here described according to the classification of 2016 [8]) are classified based on an integrated molecular and histopathological analysis (Table1).

**Table 1. WHO grades of select CNS tumors (2016) Diffuse astrocytic and oligodendroglial tumors**. From the 2016 world Health Organisation Classification of tumors of the Central Nervous System: a summary **[8]**.

| Diffuse astrocytic and oligodendroglial tumors | Grade |
|---|---|
| Diffuse astrocytoma, IDH-mutant | II |
| Anaplastic astrocytoma, IDH-mutant | III |
| Glioblastoma, IDH-wildtype | IV |
| Glioblastoma, IDH-mutant | IV |
| Diffuse midline glioma, H3K27M-mutant | IV |
| Oligodendroglioma, IDH-mutant and 1p/19q-codeleted | II |
| Anaplastic oligodendroglioma, IDH-mutant and 1p/19q-codeleted | III |

Histologically, lower (I-II) grade gliomas are characterized by tumor cells diffusely infiltrating in normal tissue, with signs of cellular and/or nuclear atypia, but normal mitotic rate (Figure 1). Oligodendroglioma frequently also includes calcification.

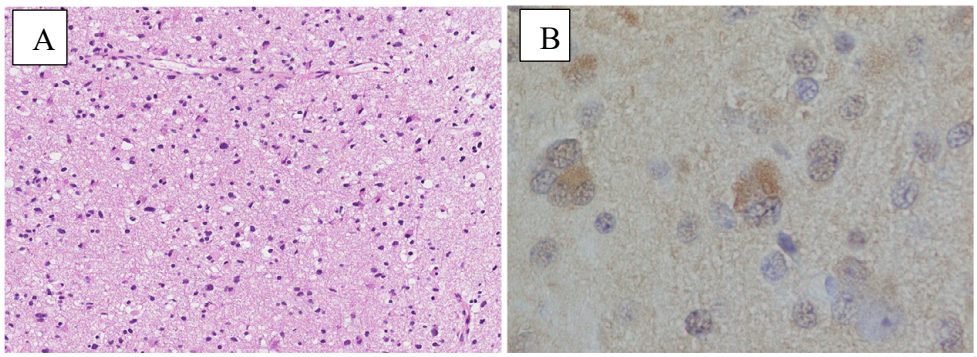


**Figure 1.A Diffuse astrocytoma grade II. Overview; HE**. Mainly monomorphic cells. *E. Englund, Department of Neuropathology, Lund, sweden.*
**Figure 1.B Diffuse astrocytoma grade II. Higher magnification; anti-hTERT-ab.** Sparse hTERT positive staining**.** *A. Rydelius, Department of Neurology, L. G. Salford and G. Skagerberg, Department of Neurosurgery, Lund, Sweden..*

In addition to cellular atypia, higher grade malignant glioma, such as anaplastic astrocytoma and oligodendroglioma grade III, show a higher rate of mitosis. The rate of mitosis may be quantified by analysing the Ki-67 protein/MIB-index (which is present during all active phases of the cell cycle including the G1, S, G2, phases and mitosis, but is absent in resting cells and usually examined by monoclonal antibody (MIB)-based immunohistochemistry) and the presence of nuclear pleomorphism.

In grade IV glioblastoma generally, a heterogenic cell pattern is seen with increasing nuclear atypia and mitotic rate and the presence of pathological vascular endothelial proliferation, which leads to a disruption of the blood-brain barrier. A central tumor necrosis, surrounded by “palisading” tumor cells, is common. (Figure 2.)

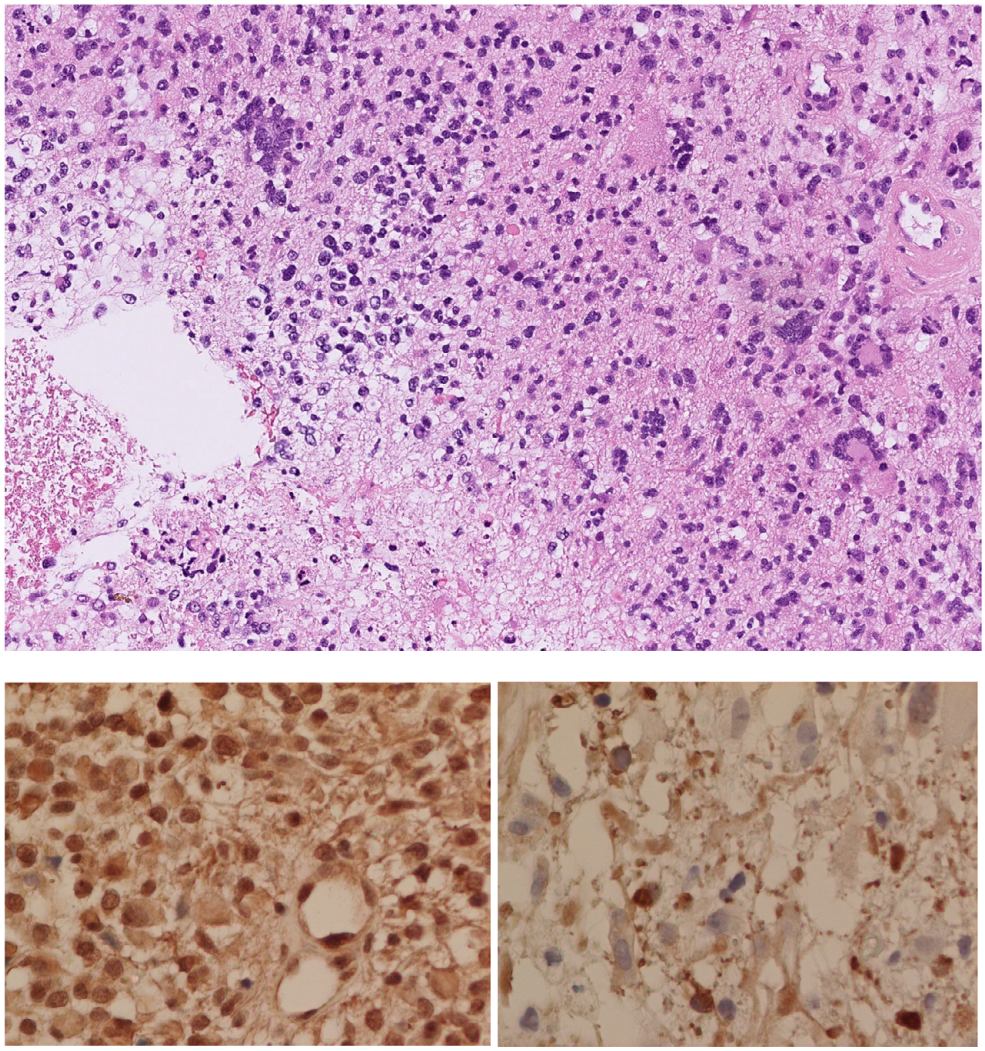

**Figure 2. Above: Glioblastoma grade IV; Overview, HE**, higly pleomorphic cells and nuclea, including giant cells, necrotic parts and a pathological vessel, *E. Englund*. **Below: Glioblastoma grade IV Higher magnification anti-hTERTab** , positive anti-TERT staining, cell dense and cell sparse parts. *A. Rydelius, L.G. Salford, G.Skagerberg*.

**Table 2. Criteria of WHO 2016** for the diagnosis of Glioblastoma grade IV, from The 2016 World Health Organisation Classification of Tumors of the Central Nervous System: a summery [8].

| | IDH-wildtype glioblastoma | IDH-mutant glioblastoma |
|---|---|---|
| **Synonym** | Primary glioblastoma, IDH-wildtype | Secondary glioblastoma, IDH-mutant |
| **Precursor lesion** | Not identifiable; develops de novo | Diffuse astrocytoma<br>Anaplastic astrocytoma |
| **Proportion of glioblastomas** | ~90% | ~10% |
| **Median age at diagnosis** | ~62 years | ~44 years |
| **Male:Female** | 1.42:1 | 1.05:1 |
| **Median length of clinical history** | 4 months | 15 months |
| **Medial overall survival**<br>**Surgery + radiotherapy**<br>**Surgery + radiotherapy+ chemotherapy** | <br>9.9 months<br>15 months | <br>24 months<br>31 months |
| **Location** | Supratentorial | Preferentially frontal |
| **Necrosis** | Extensive | Limited |
| ***TERT*-promoter mutations** | 72% | 26% |
| ***TP53* mutations** | 27% | 81% |
| ***ATRX* mutations** | Exceptional | 71% |
| ***EGFR* amplification** | 35% | Exceptional |
| ***PTEN* mutations** | 24% | Exceptional |

In the summer 2021, a summary of the most recent updated 2021 WHO classification of Tumors of the Central Nervous System (WHOCNS5) was published [9], which will lead future diagnostic criteria. One of the most important changes concerning glioma is the separation in diffuse gliomas of "adult-type" from those occurring primarily in children, called "pediatric-type", which recognizes the clinical and molecular distinctions between those diffuse gliomas that primarily occur in adults and those that occur primarily in children. Another important difference compared to the 2016 classification is the separation between astrocytoma IDH-mutant grades 2-4 and glioblastoma IDHwt grade 4, where glioblastoma grade 4 now is defined by being IDHwt, never mutant, meaning that glioblastoma IV according to earlier WHO classification with IDH mutation would now instead be classified as astrocytoma grade grade 4, IDH mutant, Tables 3, 4.

In the 2021 edition, the arabic numbers for malignancy grade are have been replaced by roman numerals.

**Table 3. A summarizing scheme of WHO grades of selected glioma types;** Adult-type diffuse glioma from Louis et al., 2021 [9]

| WHO Grades of select glioma types | Grade |
|---|---|
| Astrocytoma, IDH-mutant | 2, 3, 4 |
| Oligodendroglioma, IDH-mutant, and 1p/19q-codeleted | 2, 3 |
| Glioblastoma, IDH-wildtype | 4 |
| Diffuse astrocytoma, *MYB*- or *MYBL1*-altered | 1 |

**Table 4. A summarizing scheme with key diagnostic genes, molecules, pathways, and/or combinations in select glioma types:** Adult -type diffuse glioma, from Louis et al., 2021[9].

| Tumor Type | Genes/Molecular Profiles Characteristically Altered |
|---|---|
| Astrocytoma, IDH-mutant | IDH1, IDH2, ATRX, TP53, CDKN2A/B |
| Oligodendroglioma, IDH-mutant, and 1p/19q-codeleted | IDH1, IDH2, 1p/19q, TERT promoter, CIC, FUBP1, NOTCH1 |
| Glioblastoma, IDH-wildtype | IDH-wildtype, TERT promoter, chromosomes 7/10, EGFR |
| Diffuse astrocytoma, MYB- or MYBL1-altered | MYB, MYBL1 |

The studies presented in this thesis were conducted in patients included during 2012-2019, the histopathological diagnosis is therefore classified according to the WHO classifications of 2007 and 2016 respectively. This also includes criteria for determining management choice of the studied patients.

## Genetics

At present, the exact genetic background of glioma is not fully understood, but may be assumed to be influenced by multiple factors. These could be summarized as the neoplastic transformation of stem cells and more mature cells of glial origin, influenced by oncogenes and tumor suppressor genes, leading to dysregulation of cell division, cell-cycle arrest and apoptosis [10]. It is known that low grade glioma gradually with time transform into more malignant forms and finally into glioblastoma, which has been described as *secondary* glioblastoma [11] [12]. This is in contrast to so called *de novo* glioblastoma which seems to rapidly develop directly into this most malignant form. In the 2021 WHO classification of CNS tumors, only glioblastoma de novo is defined as glioblastoma, since the genetic profile, median age at diagnosis and the prognosis are found to be different between these entities, see above Tables 2 and 3.

In glioblastoma, the following gene alterations are most frequently observed: chromosome 7 gain, chromosome 10 loss, EGFR (epidermal growth factor) amplification and the presence of TERT (telomerase reverse transcriptase) -promoter mutation. Tumors with methylation of the O(6)-Methylguanine-DNA Methyltransferase (MGMT)-promoter, an epigenic factor associated with increased sensitivity to alkylating chemotherapeutic agents, are prognostically favourable [13].

In the 2021 WHO classification, tumors classified as glioblastoma are, as mentioned IDH1/2 *wildtype (wt)*, which means no mutation of the isocitrate dehydrogenase 1 or 2 (IDH1/2) gene, encoding for enzyme of the anaerobic glucose metabolic pathway. This is in contrast to lower grade astrocytomas, usually harbouring IDH1/2 mutations. IDH1/2 mutations are also associated with more favourable prognosis, this is also the case in tumors with a histopathological pattern with more aggressive features, thus indicating an origin of lower malignance grade. Therefore, tumors earlier classified as secondary glioblastoma, usually with IDH-mutation, are now in

the 2021 classification defined as astrocytoma grade 4, IDH 1/2 mutant. On the other hand, tumors with histologically low grade astrocytoma pattern if IDH 1/2 wild type, with additionally either TERT promoter mutation, EGFR amplification and/or chromosome 7 gain/10 loss, should be classified as glioblastoma grade 4 [14], this is relevant since these molecular patterns go with worse prognosis, similar with histological glioblastoma grade 4.

The abovementioned mutations are also assumed to have influence on tumorigenesis, thus indicating differences in origin between low grade glioma and glioblastoma 4[15].

By definition, according to both the WHO 2016 and WHO 2021 classification, in astrocytomas grade 2-4, ATRX and TP53 mutations are most frequently present and are correlated with prognosis [16]. In oligodendroglioma, per definition, loss of heterozygocity/codeletion of chromosomes 1p/19q is mandatory, unless unknown, in which case they are described *as not otherwise specified* (NOS).

With the rapid development of gene therapeutics in the field of oncology, as possible targets specific intratumoral gene aberrations can be detected. For example, therapeutic targets such as the v-raf murine sarcoma viral oncogene homolog B (BRAF) have been identified in individual malignant glioma patients [17]. This rises the hope of genetic individualized therapy, so called precision oncology. Thereby an increasing use of next generation sequencing (NGS) of tumor tissue, as part of the diagnostic procedure may be expected [18,19] using cancer comprehensive genomic profiling (CGP), for the identification of specific targets, including treatable aberrant gene expressions, in order to offer individual targeted therapy.

# Clinical features at presentation

Initial symptoms of glioma may develop gradually and increase in severity within weeks to months. The most commonly described symptoms are headache, which could be related to increased intracranial pressure, and focal motor and sensory symptoms, visual impairment, fatigue and change in mood and personality traits [20,21]. Epileptic seizures occur in 50% of glioblastoma patients during the disease and in 90% of lower grade glioma patients have symptomatic epilepsy. Diffuse sensation of gait imbalance, vertigo and extrapyramidal symptoms are frequently reported and observed.

# Radiological features and primary diagnostics

Typical radiological features of glial brain tumors are usually evaluated using diagnostic MRI. Standard sequences include three dimensional (3D) T1-weighted images (T1w) without and with the paramagnetic Gadolinium (Gd) contrast agent to detect disruption of the blood-brain barrier, axial T2- weighted images (T2w), including T2- fluid attenuated inversion recovery (T2-FLAIR), diffusion-weighted images (DWI) and perfusion MRI (pMRI) [22,23]. These methods are further described below in the section Neuroimaging in brain tumor.

Low grade glioma, e.g. diffuse astrocytoma, are characterized by a diffusely infiltrative pattern, best seen on T2w as hyperintensities, on T1w images hypointense and usually show no contrast enhancement on contrast T1w images (Figure 3 A-B). Oligodendroglioma , in addition, frequently have calcifications seen as hypointensities on T2 and hyperintensities on T1 images, but better visualized by computer tomography (CT), oligodendroglioma may also have a varying degree of vascularisation with signs of contrast enhancement on postcontrast T1w images.

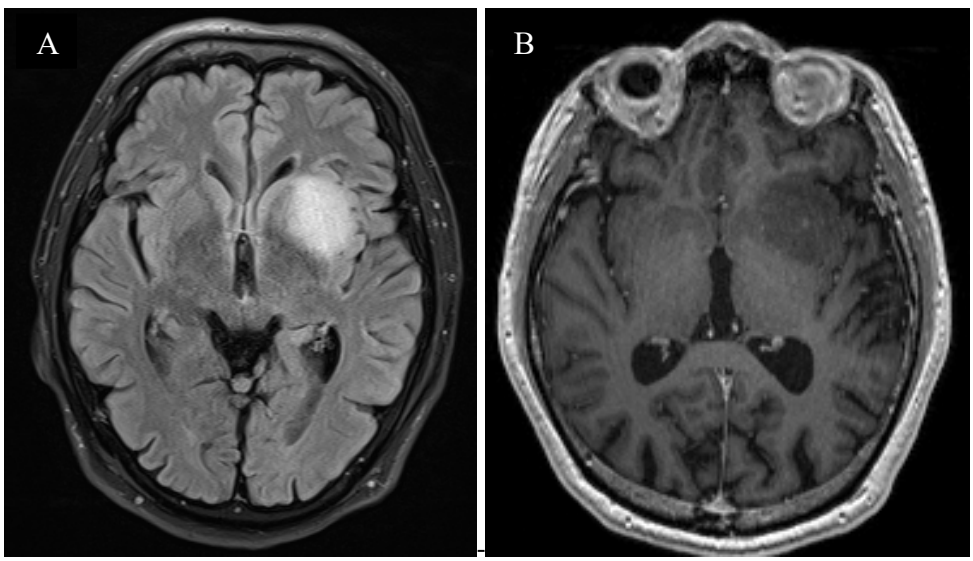


**Figure 3.A-B** (A) Axial FLAIR in a Diffuse Astrocytoma WHO grade II (B) Axial T1w with i.v. gadolinium contrastT2/FLAIR demonstrate a well described non-contrast enhancing tumor in the left insula.

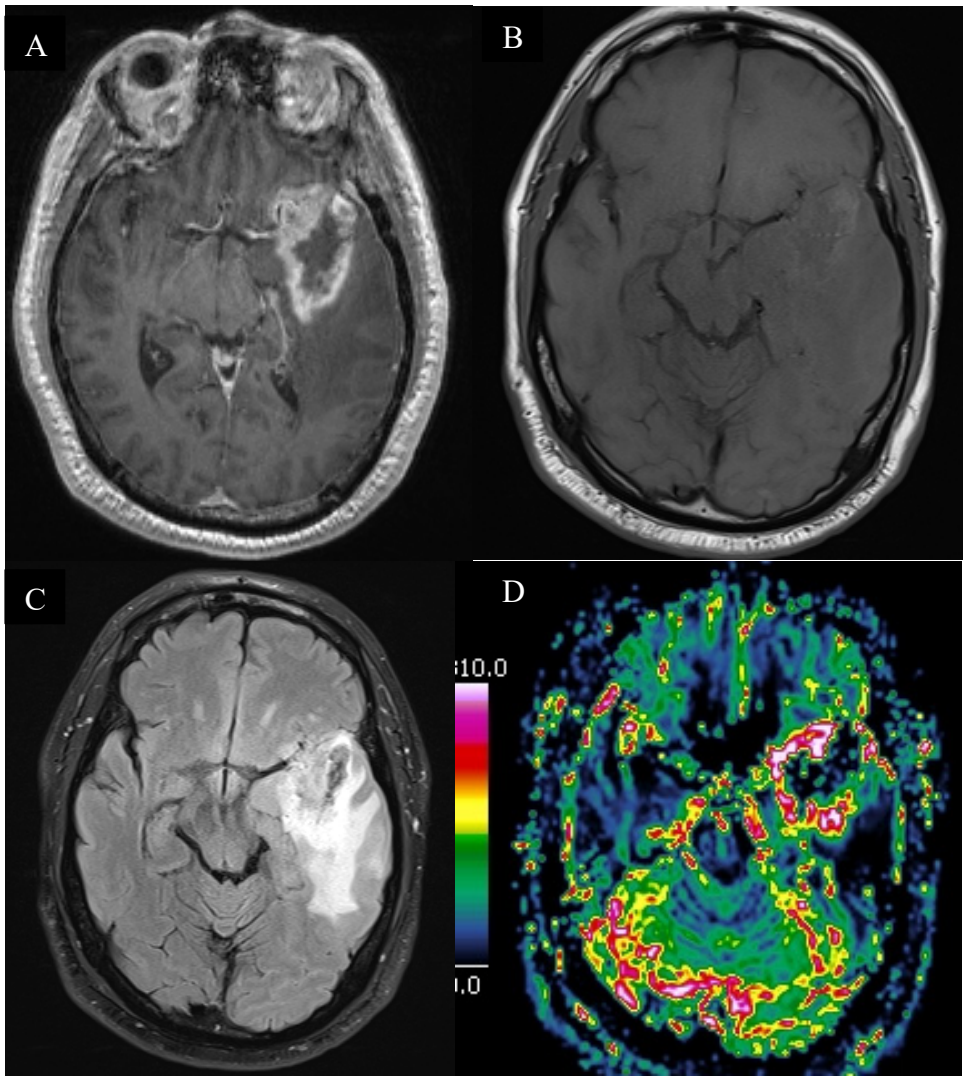


**Figure 4 A-D)** Glioblastoma WHO grade IV. Axial T1w with (A) and without Gd contrast (B), /FLAIR (C) and perfusion MRI (pMRI) (D). Demonstrate a heterogenous contrast enhancing tumor (A) with a cystic/necrotic center (A) and surrounding peritumoral vasogenic oedema (C) in the left temporal lobe. The pMRI (D) demonstrate increased cerebral perfusion in the contast enhancing parts of the tumor.

Malignant glioma, especially glioblastoma WHO grade IV, have an expansive growth, leading to compression of surrounding tissue and ventricles and midline shift. High vascularity, including signs of atypical vessels, was early described [24]. Typically, a ring of hyperintense contrast enhancement on contrast T1 images is surrounded by pronounced vasogenic oedema, best seen on T2w and T2-FLAIR images (Figure 4.A-D). The glioblastoma tumor usually contains a liquidized

central necrosis and generally has a heterogenic pattern including portions of haemorrhages, seen as T1 hyperintensities and T2 hypointensities. High cellularity, typical for malignant gliomas, reduces diffusion and may be visualized as reduced diffusion on DWI. Perfusion weighted MRI (pMRI), most frequently used dynamic susceptibility contrast (DSC), including measurement of the relative cerebral blood volume (rCBV), gives information of the tumor vascularisation. The signs of perfusion are associated to malignancy grade including IDH-mutation status, with trends to higher vascularisation and rCBV in IDH-wildtype including glioblastoma, compared to IDH-mutated tumors. At follow up, pMRI may aid to distinguish tumor progression from treatment effects, especially in high grade glioma.

Further advanced MRI such as diffusion tensor imaging (DTI), functional MRI (fMRI) and MR-spectroscopy (MRS) are not included in routine primary diagnostics and monitoring of CNS-malignancies, but may aid improved diagnostics, surgical planning and evaluation of treatment effects, further described in the section: Neuroimaging; advanced MRI.

Advanced MRI may also be used for the investigation of differential diagnosis such as CNS metastatic disease of other malignancies, cerebrovascular infarct, inflammatory or infectious processes.

# Cognitive symptoms

Cognitive function may be summarized as the ability of the individual to integrate complex information in practical life situations and in the long-term to reproduce this information when required. The cognitive function is dependent on a complex interaction of various brain structures including prefrontal and temporal cortex, white matter with corpus callosum, limbic system and cerebellum.

Brain malignancies, such as gliomas, are themselves known to cause cognitive symptoms, which may be part of the presenting symptoms before diagnosis. Cognitive symptoms are likely to appear in brain tumor patients at some stage of the disease [25]. Most commonly reported are impairment in attention, executive function and memory functions [26,27]. These symptoms may have major influence on patient autonomy and quality of life and frequently directly or indirectly on the situation of the patient's family and caregivers. A correlation between cognitive function at diagnosis and prognosis in high grade glioma has previously been described [27] and confirmed [28,29]. In a recent study of glioma patients preoperative cognitive function and final postoperative diagnosis, including tumor IDH-status, showed correlation with more impairment of verbal memory in patients with more malignant tumors [30], which was also visualized by diffusion tensor imaging (DTI) with increasing DTI changes in normal-appearing white tissue in patients with higher malignancy grade and as compared to healthy controls.

Cognitive impairment is also related to which hemisphere [31] the tumor is located. Additional deterioration in cognitive function may early indicate tumor progression [32,33], before radiologically visualized as structural change.

Antitumor treatment per se may affect the cognitive function. The influence of initial surgery [34] is usually transient, more extensively the effect of oncological therapy, especially radiotherapy has continuously been studied [26,35,36]. Negative effects of radiotherapy on cognitive function were early described, especially in children and are further commented below under treatment effects. Symptomatic medication such as antiepileptics and long-term use of corticosteroids may negatively influence the cognitive function. On the other hand preventing epileptic seizures and tumor oedema may improve the patient's cognitive function.

# Treatment of glioblastoma

Despite decades of most extensive efforts in research and technical development there is, as of today, no curative therapy to glioblastoma. The treatment at hand of glioblastoma patients aim primarily to limit tumor growth, to prolong overall survival and progression-free survival and to maintain or improve good quality of life and to relieve clinical symptoms.

## Surgery

The first step for final diagnosis and therapy of glioma is surgical debulking or total resection if feasible, otherwise biopsy. It has been demonstrated that the degree of resection influences patient outcome, but this must be carefully balanced with the risks of inflicting neurological sequelae. Improved surgical techniques, such as the use of microsurgery, MRI-guided neuro-navigation, intraoperative awake monitoring, perioperatively use of 5-aminolevulinic acid (ALA-5) fluorescence guidance [37] and the increasing use of intraoperative MRI, have aided safe debulking surgery and increased extent of resected tumor volume (EOR, %), [38] including non-enhancing tumor areas. These improvements in surgical techniques and tools have reduced postoperative risks of neurological and cognitive sequelae and, at the same time, reduced neurological symptoms due to tumor compression of healthy brain tissue. The extent of resection degree of surgery (EOR) is known to influence patient survival with a positive correlation between extent of resected tumor volume and survival [39-41]. Lately, the definition of absolute postoperative residual tumor volume (RTV) measured in mL is increasingly used as volumetric entity for quantitative evaluation of optimal surgical resection. For glioblastoma, contrast enhancing tumor parts are measured most commonly, but there is an awareness of the presence of tumor cells also in the surrounding non-enhancing T2-hyperintense regions.

**Oncological therapy**

Since 2005 standard postoperative therapy for adult glioblastoma patients consists of combined radiochemotherapy according to the Stupp-protocol [42]. This includes radiotherapy (RT) by photons 60Gy/30 fractions, with 2 Gy/fraction, with concomitant chemotherapy with the alkylating agent temozolomide (TMZ) 75mg/m$^2$, followed by six cycles of adjuvant temozolomide 150-200mg/m$^2$ 5 days every four weeks.

The techniques of radiotherapy are continuously being improved by focusing tumor target volumes (TV) and sparing adjacent presumed healthy tissue and defined organs at risk (OAR). In most publications on outcome in glioma patients after photon radiotherapy, techniques employed were 3D-conformal radiotherapy (3D-CRT) and more recently intensity modulated radiotherapy (IMRT), less studied are the effects of more novel techniques of arc-based rotation therapy such as helical tomotherapy (HT) and volumetric-modulated arc therapy (VMAT) [43,44].

Concomitant radiotherapy with TMZ has led to an increase in patient median overall survival with a few months, to presently 15 months and a small, but increasing proportion of glioblastoma patients surviving >5 years [45], compared to earlier standard therapies. It is known that MGMT-methylation status, as further described above under genetics and below under prognosis, on a group level influences the treatment effect of TMZ and the prognosis. Therefore, modifications of the Stupp protocol including the additional prognostic factor of age have been studied by Malmström et al [46]. In that study, an adaption of the Stupp protocol was suggested, based on the MGMT status (patients >60 years receiving 6 cycles monotherapy TMZ if MGMT-methylated, RT monotherapy of 34 Gy/10 fractions if MGMT-non-methylated; the shorter radiotherapy schedule was better tolerated than standard 60 Gy/30 fractions)[46]. As a further modification of the Malmström study, Perry et al [47] added that >65 years old patients with MGMT methylated tumors benefit from 40 Gy/15 fractions with concomitant and adjuvant TMZ. In patients with MGMT non-metylated tumor and >65 years old, radiotherapy with 34 Gy/10 fractions monotherapy is recommended. These protocols are better tolerated therapy schemes in the group of elder or fragile patients, or for patients with large tumor limiting full radiation dose and have gradually been implemented in the clinical setting.

Despite enormous research efforts, no additional postoperative therapy has clearly demonstrated increasing survival in glioblastoma until in 2017, when the introduction of tumor treating fields (TTFields) [48] was added in combination with the Stupp protocol. The TTFields treatment is described to affect dividing tumor cells such as glioblastoma cells in mitosis, by alternating electric fields via transducer arrays applied to the scalp. Adding TTFields to standard therapy is expected to prolong glioblastoma patient overall survival by 4 months and PFS (progression-free survival) by 2-3 months. This is according to a randomized study,

where the TTFields device was used daily during in median 8 months after terminated radiotherapy together with standard therapy of TMZ [48]. The practical use requires at least 18 hours daily, or 75% over time with the TTFields device actively in place. The high cost and the logistical and practical use and burden to the patients are limitations of this therapy, which otherwise have little known side effects.

## Symptomatic treatment

Symptomatic medical treatment and support are of great importance [49]. This includes identifying symptoms of tumor oedema and equilibrate a deswelling with an adequate dose of corticosteroids, usually betamethasone or dexamethasone, and at the same time avoing steroid side effects including sleep disturbance and other psychiatric side effects, weight gain, myopathy, diabetes mellitus and osteoporosis.

Treatment of epilepsy in high grade glioma is usually managed by antiepileptic drugs, specifically levetiracetam, lamotrigine or lacosamide [50,51] are recommended, if seizures persist additional corticosteroids may be required. As with corticosteroids, lowest effective dose of antiepileptics should be used to minimise side effects, most commonly fatigue, mood disorders (levetiracetam) and, gait disturbance (lacosamide). To achieve a satisfying response, a combination may be needed, but drug interactions must be considered.

The importance of rehabilitation is receiving increasing attention also in scientific reports [52], and should be a continuous, integrated part of therapy throughout the patient's disease, including support and adaptation to change in motor, cognitive and speech functions and generally in activities of daily living (ADL) functions [53,54].

Psychological discomfort, alteration of mood and sleeping disturbance are common and important to address [55]. It is important to identify insomnia in patients who require corticosteroids, where sleeping medication such as zopiclone or zolpidem may help stabilizing mood affection caused by lack of sleep. Other psychiatric side effects of steroids, including mood disorders and psychosis, are essential to identify. Lowest effective steroid dose is primarily recommended before symptomatic medication. If anxiety or depression are identified, supportive care with psychosocial interventions is important. Additionally, medical mood stabilisation with antidepressants may be indicated, where citalopram and low dose mirtazapine are used in clinical practice [56]. However no randomised controlled studies on anti-depressive medication specifically in brain tumor patients are currently available.

## Treatment effects

The modalities of tumor therapy in glioblastoma patients are fraught with different symptoms and side effects, here described related to given tumor therapy and in particular to radiotherapy.

*Surgery*

The surgery for diagnosis and debulking may cause neurological deficits [57], which often, and to some extent, are transient and rehabilitation may improve patient postoperative function. Small transient peri-tumoral areas of brain ischemia is not an uncommon sequelae [58]. Notably, surgery frequently reduces preoperative tumor related epilepsy and sometimes also other neurological and cognitive symptoms [34,59].

*Chemotherapy*

The most common side effects of chemotherapy are symptoms of nausea, fatigue and bone marrow supression. It therefore requires precise monitoring also of patients' general clinical status, which frequently correlates with increasing fatigue in situations of bone marrow depletion, such as neutropenia or thrombocytopenia. Thrombocytopenia per se may cause intracranial and intratumoral haemorrhages with sudden and increasing neurological deficits as clinical signs.

It is on the other hand known that efficient chemotherapy may reduce tumor related symptoms, for example improved seizure control in low grade glioma patients with severe tumor related epilepsy.

*Radiotherapy*

Radiotherapy (RT) prolongs progression-free and overall survival in low- and high-grade glioma [60,61]. However, neurotoxic reactions were early observed. [62]. In addition, despite reducing the fractional dose to a maximum of 2 Gy/fraction, related side effects were present. Radiotherapy effects are usually divided into: 1. acute reactions, 2. early delayed reactions and 3. late effects [36] and are mainly considered to affect cerebral white matter, but other radiosensitive structures such as hippocampus and other parts of the limbic system and associated cortex may be influenced.

Acute radiation reaction may occur during ongoing therapy within days to weeks: clinical features of nausea, headache, increasing fatigue and progress of neurological deficits, usually responding well to deswelling corticosteroids and the symptoms usually resolve. Histopathological peri- and microvascular changes have been observed as a probable mechanism [63].

Early delayed reaction may be seen 1-6 months after RT: clinically most frequently reported as increased fatigue and deterioration of neurological symptoms.

Histopathological features of focal or diffuse demyelination, considered transient and dose dependent. As a treatment response effect, necrosis in the tumor or its vicinity may also be expected and also more complex inflammation is described in this stage [64].

Late-delayed effects emerge from 6 months to years after terminated RT and have features of local or diffuse degeneration, parenchymal atrophy with ventricle dilatation, necrosis and inflammation [36,64,65]. Clinically, progressive cognitive deficits are observed and the condition is considered irreversible.

Risks of endocrine dysfunction, due to radiation effects on the pituitary-hypothalamic axis are increasing over time [66]. Untreated endocrine dysfunction may contribute to cognitive symptoms and the condition is probably underdiagnosed [67].

Cognitive influence of RT as a late occurring effect is sparsely studied in glioblastoma patients, because of the severe prognosis, with short overall survival. Long term effects in children and adults with low grade gliomas over time are described as a gradual deterioration of attention, memory, psychomotor function and executive functions [68,69].

*Mechanisms and specifically vulnerable regions:* A cascade of reactions induced by radiotherapy in the irradiated brain beyond the tumor tissue, including oxidative stress, vascular damage, demyelination and inflammation, has been described [70]. White matter changes are frequent, including demyelination. The hippocampus, as part of the limbic system and crucial for memory function, is the most studied brain structure [71] concerning radiotoxic effects in the brain. Efforts to spare hippocampus with results from the RTOG 0933 trial are promising concerning hippocampus sparing whole brain radiotherapy (WBRT) [72], but it is still unknown if they are sufficient to prevent long term cognitive decline after RT.

*Typical radiological findings:* Radiological signs of radiotherapy effects mainly seen on white matter are periventricular changes, in addition focal and diffuse changes of signal intensity of white matter but also diffuse loss of structure of white matter and adjacent gray matter, atrophy and subsequently hydrocephalus [36]. The specific neuroradiological findings of radiotherapy reactions and other treatment reactions are further described below in the section neuroimaging in brain tumors, Imaging effects of radiotherapy.

*Importance, obstacles and limitations in evaluation of treatment effects:* To evaluate the effects of therapy is crucial for the wellbeing of glioblastoma patients. However, the mode of evaluation in literature is very heterogenic both methodologically, e.g, which cognitive test-methods used, exact patient diagnosis, the time points chosen for evaluation and the frequency of repeated evaluation. The rapid technical development in the field of imaging adds new opportunities to identify and follow up treatment effects. Additionally, which structures of the brain investigated as

being vulnerable for damage vary, as do the imaging methods of assessment and the microstructural focus of these. This all makes the comparison of treatment effects challenging, highlighted in several reviews [34,35]. In this thesis, studies of early predictive imaging methods and longitudinal treatment effects were approaches.

## Prognosis, survival and clinical prognostic factors

The prognosis in glioma patients vary depending on histopathological grade, where low grade glioma WHO grade I-II have a more favorable prognosis of 5-15 years overall survival, depending on age, tumor size at diagnosis and given postoperative therapy. With increasing malignancy grade, the prognosis deteriorates, with a median survival of 2-5 years in anaplastic oligodendroglioma III, 1,5-3 years in anaplastic astrocytoma III, and 15 months in glioblastoma IV, even after standard treatment according to Stupp. Age plays a major role on survival in glioblastoma, as do clinical performance status [1,60] (ECOG/WHO/Z/UBROD/ or Karnofsky) and cognitive function at diagnosis. The molecular feature of the tumor, if MGMT promoter methylation is present or not, has impact on the expected response on the postoperative therapy with radiotherapy, concomitant TMZ and lately TTFields [13,48,73,74] . The initial degree of surgical resection and whether reoperation at tumor recurrence can be offered and performed, also influence survival [75,76]. For these decisions, tumor location; if eloquent or not, and tumor size at diagnosis play important roles, also highlighting the prognostic importance of discovering the tumor early [75,77,78].

# Neuroimaging in brain tumors


## Radiologic evaluation in clinical practice

*Magnetic resonance imaging MRI* constitutes the golden standard of imaging of suspected malignant brain tumor, although radiological examination is often initiated with a semi acute CT-scan.

*T1 weighted MRI sequences (T1w).* The routine MRI protocol includes 3D-T1w MRI. It should be performed before and after administration of intravenous gadolinium (Gd) contrast agent, in order to evaluate the integrity of the blood brain barrier. T1 sequences, usually including so called magnetization-prepared rapid acquisition gradient echo (MPRAGE) contrast enhanced sequences, provide precise images of the anatomical situation including presence of expansive signs with compression of cerebrospinal space/ventricles, or suspected necrosis. Tumors are usually hypointense (dark) on T1 image before contrast. Higher grade glioma usually has a heterogenic signal pattern with contrast enhancement and glioblastoma has a typical ring-like contrast enhancement surrounding a central necrosis (Figure 4.A). Haemorrhagic parts are common and appear hyperintense in the pre-contrast T1.

*T2 and T2 -fluid-attenuated inversion recovery (FLAIR).* T2 weighted images can identify oedema and tissue morphology abnormalities, including white matter changes, and tumors appearing as hyperintensities/brighter, including low grade tumor structures which are not contrast enhancing e. g. have preserved blood brain barrier. Additionally, in T2-fluid-attenuated inversion recovery (T2-FLAIR) sequences, the cerebrospinal fluid is nulled and therefore appear dark, in contrast to structural tumor changes and tumor edema which appear bright. By T2 and T2-FLAIR, both high grade tumors and tumors of lower malignancy grade can be identified as hyperintensities (bright) lesions (Fig 2A, 1A).

*Diffusion-weighted imaging (DWI)* measure the movement (self-diffusion) of water molecules which depends on the undelying tissue properties [79]. It is an important tool in clinical routine to detect acute ischemia, as this is a primary differential diagnosis to tumor or post-operatively due to perioperative circulation impairment. DWI is usually based on T2 weighted imaging with additional magnetic field gradients, labelling water protons. The tissue specific water diffusion gives information of tumor tissue cellularity as well as extracellular space, including tumor edema [80,81]. The rate of the diffusion is quantified by the apparent diffusion coefficient (ADC) which is usually influenced by the degree of tumor malignancy, due to increasingly densely packed cells, giving a restricted free diffusion and thereby decreased ADC values [82].

*T2 star (T2*)* sequences or susceptibility weighted imaging (SWI) are included to distinguish blood components from tumor.

## Advanced MRI techniques

Advanced MRI adds additional information in brain tumor imaging and includes diffusion tensor imaging (DTI), perfusion weighted imaging (pMRI), functional MRI (fMRI) and MR spectroscopy (MRS) [83], of which DTI is one main focus of this thesis.

*Diffusion tensor imaging (DTI)*

DTI is an advanced technique allowing measuring of both the overall diffusivity and the directional dependencies of the diffusion, and is retrieved from several different DWI images [84]. From the DTI one can extract measured values, such as mean diffusivity (MD) and fractional anisotropy (FA), the latter an index between 0 and 1, giving an estimate of how anisotropic i.e., how directionally dependent the diffusion is. For instance, a FA close to zero means isotropic diffusion (equal in all directions) whereas an FA closer to 1 is highly directionally dependent and can be seen in corpus callosum, where the axons are densely packed and insulated in myelin sheaths. DTI has been proven especially suitable to analyse the microstructure of the tightly packed white matter as well as giving an indication of different types of

tissue damage, especially in white matter, such as demyelination and axonal damage [85] [86,87].

The diffusivity can further be divided into axial diffusivity (AD) and radial diffusivity (RD). AD represents the diffusion along the preferred diffusion directions, along the white matter fibres, whereas RD estimates the diffusion perpendicular to the axons. Previous studies have demonstrated the value of DTI and tractography in pre-surgical planning as well as in post-surgical follow-up. [88,89].

*Perfusion weighted imaging (pMRI)* illustrates the active vascularisation in tissue, where dynamic susceptibility-weighted contrast enhanced (DSC) or dynamic contrast-enhanced (DCE) perfusion MRI using iv bolus of Gd are most relevant for brain tumor analysis, by measuring relative cerebral blood volume (rCBV) and relative cerebral blood flow (rCBF) [90]. rCBV is normally only visually elevated in vessels, but not in normal healthy brain parenchyma, neither in tumor necrosis nor in treatment effect related so called pseudoprogression areas. Viable high-grade glioma tumor tissue usually has elements of neovascularisation/angiogenesis, which will show increased perfusion rCBV. However, the resolution of pMRI is limited and lesions smaller than approximately 10 mm cannot be well identified on DSC sequences. pMRI may be used as a tool for preoperative diagnosis [91] of tumor grade. Recent studies strongly suggest always to include perfusion weighted imaging in the pre-surgical, and follow-up examinations of patients with brain tumors [22].

*Functional MRI (fMRI)* measures active tissue structures in real-time during a specific task, activated structures thereby demonstrate elevated blood perfusion due to increased oxygen demand (blood oxygenation level-dependent (BOLD) effect) [92]. See also pre-operative evaluation.

*MR spectroscopy (MRS). MRS* measures the metabolism in tissue in predefined volumes of interest, by the spectral absolute concentration or by the spectral ratios of different metabolites [93]. Choline (Cho) increase illustrates increased turn-over of cell-membranes, expected to be elevated by increased tumor cell activity and could indicate higher malignancy grade, whereas if detected, lactate correlates with hypoxia which may indicate tumor invasion and malignant vessels. Lipids reflect necrosis. Generally, elevated Cho, lactate and lipid levels may be associated with increasing malignancy grade, whereas the neuron associated N-Acetyl Aspartate (NAA) tends to decrease in concentration with increasing malignancy grade [94,95]. Other metabolites currently studied are Creatine (Cr), Myo-inositol (Ins) and Glutamate/Glutamine (Glx) [94]. MRS may also aid differentiating a cystic or solid high-grade tumors from metastases if considered a pre-surgical issue in the clinical setting [94-96]. Also, recent MRS studies demonstrate the utility of MRS to define IDH and hTERT mutant gliomas as indicator of degree of malignancy [97,98]

MRS technique is not yet an established method in clinical routine practice but is studied in research settings as a complement both for diagnostics and for therapy monitoring [95].

*Multimodular combination of advanced MRI* including pMRI and MRS techniques in the preoperative diagnostics seem to be increasingly favourable to differentiate tumor types [99,100] and for postoperative therapy planning.

## Preoperative evaluation for diagnosis

In addition to the routine conventional T1 including pre- and post-contrast and T2 weighted MRI, sequences including diffusion and recommended perfusion weighted imaging performed for pre-surgical assessment, some additional sequences may be valuable for pre-surgical treatment planning.

Functional fMRI [92]and tractography by DTI [89] are additional methods used for preoperative planning in order to minimize per-operative damage to the brain and reduce the risk of postoperative neurological sequela. These sequences allow for instance evaluation of language lateralisation, visual and motor function as well as potential influence on the white matter tracts, information valuable for the neurosurgeon concerning risk evaluations in the planning prior to surgery.

*Tumor volume assessment:* Tumor volume assessment in clinical preoperative practice is often made by measurement of the contrast enhanced diameter product, measuring width (w) length (l), and height (h), calculating the tumor volume:

$$V = w \times l \times h \times 0.5$$

## Postoperative evaluation

Postoperative evaluation is performed by MRI and includes T1-weighted imaging before, and after administration of contrast agent T1-w by MPRAGE, T2-w and FLAIR and DWI, which should be performed within 48 hours to assess the degree of resection and to identify possible complications to surgery such as ischemia or haemorrhages [22].

Residual postoperative tumor volume is also usually determined by comparison of the contrast enhanced cross diameter product. However, quantitative volumetry assessment by semiautomatic techniques is gaining increasing importance [22,75], as seen from work presented in thesis.

When MRI is performed later than 48 hours postoperatively, pMRI may aid to identify vascularity as a measurement of viable tumor in contrast to reactive postoperative inflammation.

Multimodular combined advanced MRI with DTI, PWI and amino acid PET such as 18F-fluoroethyltyrosine (FET)-PET in the postoperative treatment planning may improve accuracy and be valuable for further treatment decisions [99,100].

## Treatment follow up

*MR Imaging*

MRI protocol including T1-w imaging before and after administration of contrast agent, T1-w, MPRAGE, T2-w and FLAIR are the golden standard for follow up and give information of remaining contrast enhancing tumor areas [22]. But it may also show treatment-related effects such as pseudo-progression and early and late radiotherapy reactions, which cannot clearly be distinguished from tumor progression by these sequences. Cerebral perfusion techniques (pMRI) by using for example DSC, are increasingly common in clinical practice to identify tumor recurrence and to evaluate the effect of postoperative therapy and are recommended to be included in follow-up of brain tumors [101]. This complementary information is important since tumor progression tends to show increased perfusion with raised rCBV, whereas treatment reaction including pseudo-progression usually does not [101]. The rCBV result thereby influences further treatment plans. As limitation of pMRI is the rCBV resolution, limited to lesions at least of 10 mm diameter

*MR spectroscopy (MRS)* may, even if not used in daily clinical routine, provide radiological support to determine if treated tissue consists predominantly of radiation necrosis or tumor, which *per se* is an important diagnostic dilemma. Also, even if more research-oriented, an application can be to evaluate which part of a residual tumor is most aggressive for target for radiation therapy[95].

*Positron emission tomography (PET),* especially the use of $^{18}$F-fluoroethyl-L-tyrosine (FET-)PET [102,103] as a tracer of active tumor metabolism, is an additional tool to distinguish tumor progression from treatment effects. It could, as mentioned, also be used in a multi-parametric setting in the treatment planning, predicting treatment response [100], before initiated radiotherapy.

## Response assessment

*The MacDonald criteria* were the first recommendations to categorize tumor response systematically for phase 2 trials into complete response, partial response, stable disease and progressive disease, integrating steroid use and neurological symptoms [104].

Routine praxis is the comparison of the contrast enhanced cross diameter product (CDP).

*Response Assessment in Neuro-Oncology (RANO*) *criteria* were developed to define and monitor treatment response for clinical trials [105] as a further development of the previously used Macdonald Criteria. Radiological findings including measurement of contrast enhancing and non-enhancing areas derived from perpendicular diameters are integrated with clinical performance status and steroid intake. In the latest updated RANO version for surgery and tumor progression in clinical trials, additionally, limitations of original RANO such as no consensus regarding use of advanced imaging such as perfusion weighted imaging are addressed. However, by using the very first "Post-Radiation MRI Examination as the Reference for Evaluating Radiographic Response in Newly Diagnosed GBM" [106], this limitation of not mandatory perfusion examination is clearly reduced, by having a defined baseline examination later, after completed radiotherapy, minimizing the risk of misinterpretation of pseudoprogression or postoperative artefacts from true progression.

*Parametric Response Maps.* A method to investigate tumor responding properties at an early stage is the so called parametric response mapping (PRM) by the use of voxel by voxel analysis quantifying regional tumor tissue change by advanced MRI techniques such by perfusion weighted MRI (pMRI) including cerebral blood volume (CBV) [107] or by diffusion-weighted MRI. Previous studies using apparent diffusion coefficient parametric response maps (ADC-PRM), also called functional diffusion maps, have shown some promising results in patients with primary high grade gliomas treated with chemo-radiotherapy [108-110] by being predictive at 3 weeks into therapy intending to identify and predict responders to standard therapy during ongoing treatment. Specifically, higher volumes of voxel of changed diffusion from treatment start three weeks into treatment indicated stable disease after terminated treatment [108].

This method has been specifically assessed in this thesis.

## Radiological effects of radiotherapy

Imaging effects of radiotherapy influencing white matter are well described [36,111,112]. For analysis of these effects, DTI constitutes one of the most important tools, in addition to typically increased T2 and FLAIR signals [113].

A decrease of FA in white matter compared to age matched healthy controls was shown to correlate with cognitive deficits in children one year after treatment with whole brain RT [114]. White matter changes after RT appear to be dose dependent [115]; early change, mainly by increase of RD, have been demonstrated and predicted cognitive decline [116]. Specifically, anterior cingulate superficial white matter was recently observed to be dose dependently vulnerable for early DTI changes: i.e., decrease in FA and increase in RD and MD were associated with later deficits of executive function [117].

Apart from white matter, limited observations on effects of the limbic system including hippocampus have been studied by DTI [118], which gave indications of hippocampal FA decrease after RT.

Recent findings showed volume loss of other structures which may be vulnerable to radiotherapy, such as in amygdala and cortical thinning of associated cortex (including premotorcortex) and entorhinal cortex with influence on memory [119]. These structures have not yet been studied using DTI, which could potentially add important information on microstructural influence of RT.

DTI findings related to radiotherapy is a further topic of this thesis.

# Cognitive evaluation in brain tumor patients

## Commonly used cognitive test batteries

Historically, the most frequently used cognitive test in brain tumor studies is the Mini-Mental-State Examination MMSE [120]. This test was originally developed by Folstein et al. [121] for dementia syndrome diagnostics and widely used for all types of cognitive screening. However well-known, convenient and time efficient, this test is less sensitive for subtle, but relevant changes in cognitive function in brain tumor patients, and strongly influenced by premorbid cognitive function and level of education. Focal neurological symptoms due to the tumor, such as aphasia, neglect or hemianopsia, may influence test procedure by an unproportional decrease in test results and are therefore further limitations for the use of MMSE in brain tumor patients.

Relevant and frequently examined cognitive domains in glioblastoma patients are addressed by the test-batteries described in repeated time settings by Meyers [122]. Deterioration within these domains could predict radiological tumor progression by six weeks:

- Attention span, by Digit span from Wechsler [123], by repeating of numbers for- and backwards.
- Graphomotor speed: Digit Symbol from Wechsler [123], testing the ability to code symbols for numbers during measurement of time.
- Verbal memory domain: Hopkins Verbal Learning Test; HVLT; [124], testing the recall of three categories of words in the time settings of immediate testing (HTLV-recall), at distraction and after delay (HTLV-recognition).

- Verbal fluency: Controlled Oral Word Association (COWA) [125]; production of words beginning at a specific letter within predefined time.
- Visual motor speed/processing speed: Trail Making Test Part A (TMT A, from Lezak 1995 [126-128]) by letting the patient connect dots in numerical order under time measurement.
- Executive function: Trail Making Test Part B (TMT B, from Lezak 1995 [126]) where the patient is connecting alternating numbers and letters under time measurement.
- Motor speed and dexterity: Grooved Pegboard (from Lezak 1995 [126,127]) patient placing pegs in holes, measuring the examination time from the dominant and non-dominant hand separately (Pegboard right hand/left hand).

Especially the domains of executive function and attention had a strong association with prognosis, when analyzed postoperatively before patients underwent oncological treatment, using the updated versions of these test-batteries in a study by Johnson et al [27].

CNS-vital signs (CNS-VS) is a standardized, computerized cognitive test, which constitutes further development and integration of these test domains, where demographic factors such as age, length of education and sex are integrated in the test standardization [129]. It contains nine cognitive function domains:

- composite memory
- verbal memory
- visual (spatial) memory
- executive functioning
- information processing speed
- psychomotor speed
- reaction time
- complex attention
- cognitive flexibility

The results are retrieved as standard scores where 90-109 are within the range of normal cognitive function [129,130]. This test has previously been used in various neurological conditions such as CNS trauma [131], multiple sclerosis (MS), systemic lupus erythematosus (SLE) [132,133] and in brain tumor patients [134]. The algorithm allows repeated testing for comparison of cognitive function over time, without learning effects and is therefore suitable for cognitive follow up.

# Aims

General aims of this thesis have been to longitudinally follow glioblastoma patients from diagnosis radiologically by advanced MRI modalities: to study effects of currently used therapy and also patient clinical neurological and cognitive function, including quality of life assessment and to identify early prognostic factors.

The specific aims for the separate papers included in this thesis are as listed:

I. To evaluate the reliability of different methods of estimation of residual tumor volume (RTV) and extent of tumor resection (EOR) from neurosurgery, by comparing quantitative volumetric radiological assessment with: (i) subjective visual estimation; and (ii) with objective volume estimations by using a simple formula.

   The second aim was to clarify whether quantitative volumetric radiological assessment of RTV and EOR would provide accuracy in predicting progression-free survival (PFS) and overall survival (OS) in malignant glioma patients.

II. To longitudinally explore the effects of adjuvant oncological treatment, including arc-based radiotherapy, on cognitive function and to investigate if specific clinical parameters are associated with the cognitive function.

   The second aim was to explore correlations between cognitive function at baseline before adjuvant treatment and time to tumor progression and overall survival.

III. To investigate the longitudinal effects of radiotherapy (RT) in various normal-appearing radiated brain regions, measuring changes in diffusion parameters during and after modern arc-based radiotherapy methods, such as volumetric modulated arc therapy (VMAT) and tomotherapy, in patients with glioblastoma WHO grade IV, using diffusion tensor imaging (DTI).

IV. To evaluate the predictive value of mean diffusivity parametric response maps (MD-PRM) at three weeks into oncologic standard treatment on progression-free survival (PFS) and overall survival (OS) and specifically on treatment response at 8 and 12 months, in newly diagnosed glioblastoma patients after primary surgery or biopsy for final diagnosis.

Second aims were to evaluate if the degree of surgical resection influences the MD-PRM pattern and finally if there is a correlation between MD-PRM findings and the established prognostic marker of MGMT-promoter methylation.

The intention in future is to optimize the choice of surgical resection degree, to offer individualized postoperative therapy in balance with expected survival benefits in relation to expected side effects, and improved decision-making during follow up, with the goal of improving patient survival in equilibration with quality of life and clinical and psychological well-being.

# Material and methods

## Patients

**Paper I** is a retrospective study conducted from MRI findings of all patients who underwent surgery, by tumor resection at the department of neurosurgery in Lund, Sweden during 2012-15. 70 patients with the final diagnosis glioblastoma WHO grade IV according to WHO classifications 2007 [135] and with postoperative MRI performed within 72 hours, were included. Clinical data was retrieved from patient records including age, gender, the intraoperative use of fluorescence-guided surgery using 5-aminolevulinic acid (5-ALA), final histopathological diagnosis (including MGMT-status when available), Karnovsky performance status (KPS), postoperative oncological therapy and whether reoperation was performed, progression-free survival (PFS) and overall survival (OS).

**Papers II-IV** all include newly diagnosed glioblastoma patients from a cohort of 40 glioma patients as part of a prospective longitudinal MRI study performed in Lund 2013-2019. Inclusion criteria were patients aged ≥18 years, planned for standard postoperative therapy [42] with radiotherapy 60Gy/fractions and concomitant (75mg/m$^2$) and adjuvant (150-100mg/m$^2$) Temozolomide (TMZ) and patients' written informed consent. The study protocol included MR examinations, clinical and neuropsychological follow up at baseline after surgery, but prior to radiotherapy and additional predefined time points, it also included the collection of clinical and demographic data (Table 5 A).

Radiotherapy was applied by arc-based photon radiotherapy in all patients included in the study for papers II-IV with either helical tomotherapy (HT) or volumetric modulated arc therapy (VMAT).

**Paper IV**: This study specifically evaluated MRI examination for the MD-PRM analysis according to the study protocol at two time points: at baseline (w0) at the start of radiotherapy, and at three weeks into radiotherapy (w3). Both examinations were required from each study participant as final inclusion criteria.

**Table 5.A: Study protocol** including the time points for the clinical and cognitive examinations and MR imaging for patient cohort of papers II-IV

| Time point | Examinations |
|---|---|
| **Postoperatively or before RT** | MR-Protocol<br>Clinical examination<br>Neuropsychological examination |
| **3 weeks** | MR-Protocol<br>Clinical examination |
| **6 weeks** | MR-Protocol<br>Clinical examination |
| **3 months** | MR-Protocol<br>Clinical examination<br>Neuropsychological examination |
| **6 months** | MR-Protocol<br>Clinical examination |
| **12 months** | MR-Protocol<br>Clinical examination<br>Neuropsychological examination |
| **18 months** | MR-Protocol<br>Clinical examination |
| **24 months** | MR-Protocol<br>Clinical examination |

**Table 5.B. Number of patients meeting final inclusion criteria in studies II-IV** and number of patients for follow up participation

| Study paper number | Baseline examination number of patients. | 3 weeks | 6 weeks | 3.5 months | 6 months | 12 months |
|---|---|---|---|---|---|---|
| II | 31 | | | 26 | | 13 |
| III | 27 | 26 | 25 | 25 | 22 | 15 |
| IV | 31 | 31 | | | | |

For clarification of the current study cohort in this thesis papers II-1V: the patients included belong to a larger prospective, longitudinal study of patients over 18 years with suspected primary brain tumor scheduled for surgery or biopsy of their lesion and who have consented to participate (Figure 6, flowchart MR study). Whereas paper I is a retrospective MR imaging study of glioma patients prior to and post-surgery for evaluation of residual brain tumor after surgery.

The studies had ethical permits (#2011/598, #2011/814, #2012/188, #2014/368, #2016/957)

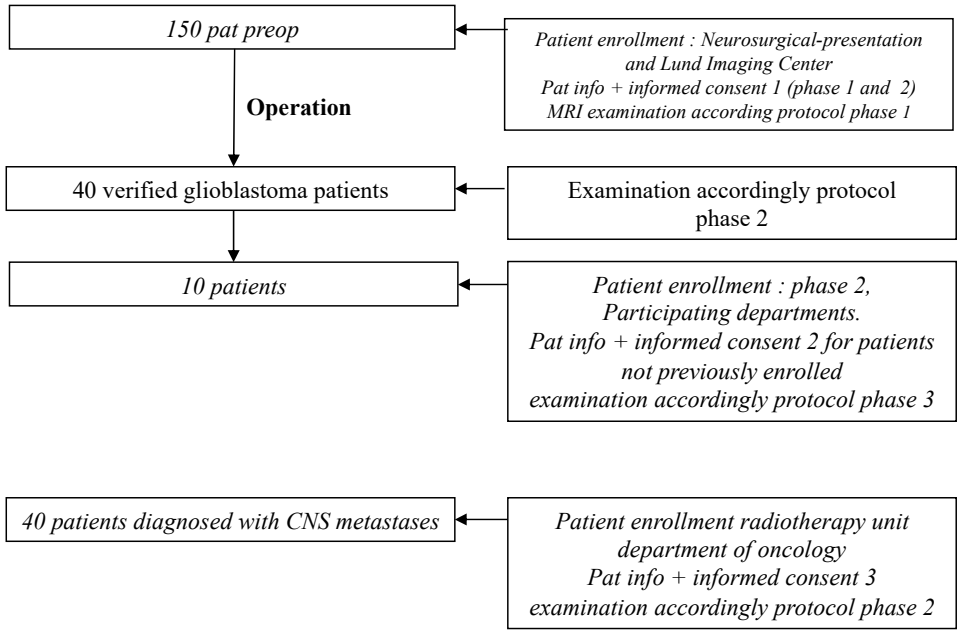


**Figure 6. Flow chart of patient inclusion criteria in the MR study cohort.** Papers II-IV describe patients investigated during phase 2 (regular) *within the framework of the prospective, longitudinal study (italics)*.

# General imaging

## Paper I

In this retrospective study, evaluations of pre- and postoperative magnetic resonance images (MRI) examinations, retrieved from the clinical routine assessment of newly diagnosed glioblastoma patients were performed. These examinations included T1weighted (T1W) MRI pre- and post-Gadolinium (Gd) contrast enhancement, T2-weighted (T2W) images, T2-fluid attenuation inversion recovery (FLAIR) clinical diffusion-weighted (DWI), 3D T1W with $1mm^3$ cubic resolution, from different 1.5 and 3.0-T MRI scanners used in clinical routine practice. Postoperative examinations were performed within 72 h following surgery.

## Papers II-IV

These studies are based on MRI data, collected from a prospective, longitudinal MRI study of patients with newly diagnosed glioblastoma (flowchart Fig. 6 ),

performed on a Magnetom Skyra 3T system (Siemens Healthcare, Erlangen, Germany). MRI was longitudinally performed (Table 5.A.), including morphological imaging; T2W FLAIR and T1W- Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE), performed before and after intravenous contrast 0.2 mL/kg of Gadolinium-DOTA (Dotarem®, Gothia Medical/Guerbet) and dynamic susceptibility contrast perfusion (DSC). DWI was acquired using 30 nonlinear diffusion-encoded directions with a b-value of 1000 s/mm$^2$ and a 2 mm$^3$ isotropic resolution.

# Pre and post processing

## Paper I.

Tumor location of each patient was identified and was classified as being eloquent (motor/sensory cortex, visual, auditory or speech center, basal ganglia, hypothalamus, brainstem and corpus callosum) or non-eloquent (non-eloquent: frontal or temporal polar location, right parietooccipital location and cerebellar hemisphere locations), based on the definition of Sawaya [136] and Lacroix [137], with the modification that near-eloquent tumors in this study were dichotomized as also being eloquent.

Volumetric assessment of contrast enhanced tumor volume in mL was performed by quantitative volumetric radiologic assessment (QRA) by an in-house developed software Evaluation-Graphic User Interface (EvalGUI) and was compared with conventional clinical subjective inspection by an experienced neuroradiologist. Only contrast enhancing tumor was defined as tumor in both conventional and QRA analysis preoperatively. Additionally, for postoperative volume measurements, the T1W MRI images without and with contrast were compared for exclusion of postoperative signal changes, blood and surgical material. For QRA, the contrast enhancing tumor areas from each examination were manually delineated pre- and postoperatively.

The diminished volume (DV) measured in mL was calculated postoperatively:

$$DV = \text{Preoperative tumor volume (PTV)} - \text{residual tumor volume(RTV)}.$$

Extent of resection (EOR) (%) was calculated as:

$$EOR = DV/PTV.$$

The results from the quantitative volumetric measurement and the reliability of those were compared with conventional measurement methods performed in two steps:

1. Subjective visual evaluation of (a) EOR and (b) RTV of the whole study cohort. The rating was performed by an experienced neuroradiologist. EOR has been rated into ≤ 88 %, 88.1-94.9%, 95-98 % and > 98 % (quartiles). RTV was divided into tertiles: 0-0.9 ml, 0.91-5.9 ml and >5.9 ml. This rating was compared to quantitative volumetric measurements QRA.

   Objective evaluation by an experienced neuroradiologist (K. A-K), measuring tumor width (w), length (l) and hight (h), then calculating the tumor volume using the diameter product-formula:

   $$Volume = w \times l \times h \times 0.5.$$

2. An independent investigator randomly chose 25 patients from the study cohort for such 3-dimentional measurements and quantitative volumetric measurements (QRA) at two different occasions with an interval of at least 4 weeks.

The intra-rater agreement (agreement between measurements at the first and second occasions) was quantified for the different methods (QRA versus the volume calculated by using the formula: w x l x h x 0.5).

## Paper III.

Diffusion-weighted images were corrected for subject motion and eddy current artifacts using ElastiX [138,139]. All MRI images were co-registered to the MPRAGE images. Diffusion tensor imaging (DTI) was performed to retrieve radial diffusivity (RD), axial diffusivity (AD), mean diffusivity (MD) and fractional anisotropy (FA) using the in-house developed MATLAB based software. Each patient's computed tomography (CT) for radiotherapy treatment plan and the corresponding dose distribution maps were co-registered to the baseline T1-MPRAGE image, using ElastiX with rigid body transformations.

Selection of regions of interest (ROI): Homogenous normal-appearing tissue which was located within the irradiated area was defined. As white matter structures, the corpus callosum and the centrum semiovale were examined, specifically ROIs within the genu corpus callosum (CC Genu), the corpus (body) corpus callosum(CC body) and the splenium corpus callosum (CC Splenium) with a ROI of 50 voxels (=0.05cm$^3$) per structure. The structures from CC were contoured on the FA color-map using the baseline examination, thereafter manually confirmed and fine adjusted, if needed, on the subsequent scans (figure 7.A). ROIs within centrum

semiovale (CSO) in the right hemisphere was contoured on the T1W image (Fig. 7.B). The ROIs in this structure comprised 350-545 voxels (=0.350-0.545cm$^3$).

Normal-appearing structures from the limbic system were chosen in hippocampus and amygdala, which were bilaterally delineated on the T1W image and manually adjusted, to exclude partial volume effects, using the MD map, comprising 300-640 voxels and 80-120 voxels respectively (0.30-0.64 cm$^3$, 0.08-0.12 cm$^3$). (Fig. 7.D,C).

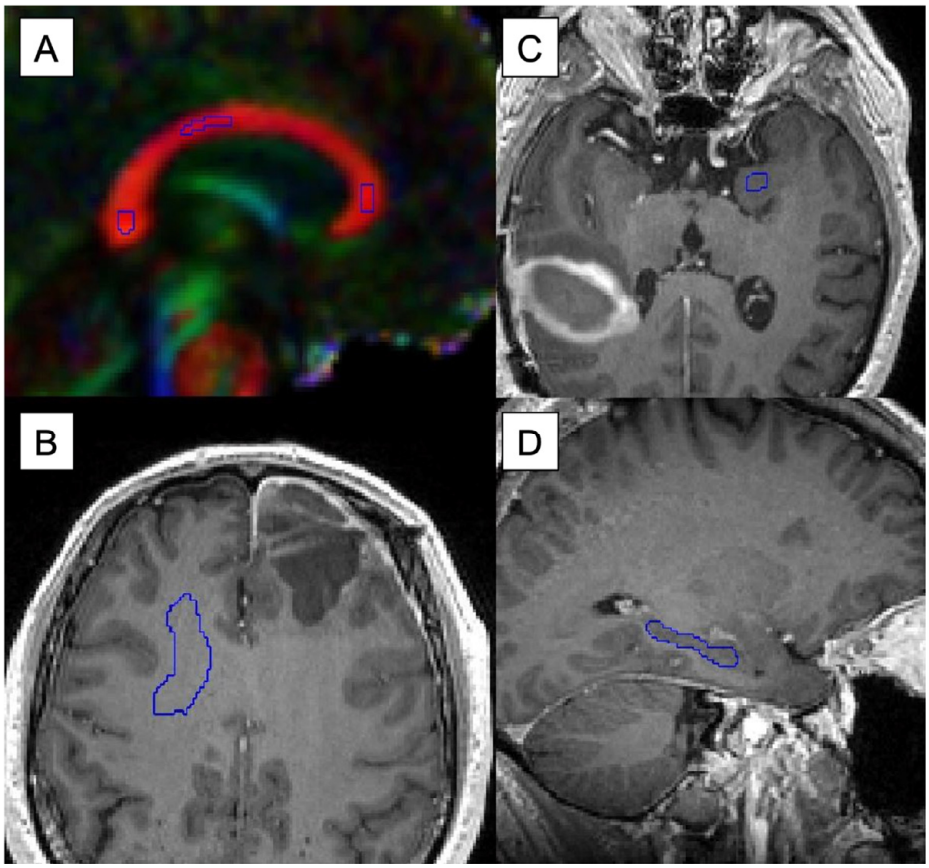


**Figure 7. Placement of Regions of interest (ROI) A.** The corpus callosum on a FA-color map. The ROI placed within the structures from left: splenium, corpus and genu, viewed at a sagittal view. **B.** The centrum semiovale in the right hemisphere on a post contrast T1-weighted image, viewed on the transversal plane. **C.** The amygdala in the left hemisphere on a post contrast T1-weighted image, viewed on the transversal plane. **D.** The left hippocampus on a post contrast T1-weighted image, viewed on the sagittal plane

## Paper IV.

The diffusion-weighted images were corrected for subject motion and eddy current artefacts using ElastiX [138]and masked using FSL BET [140,141]. A diffusion tensor imaging (DTI) analysis [86] was performed on the DWI data to obtain maps

of the mean diffusivity (MD) and the non-diffusion-weighted signal ($S_0$) using the in-house developed software based on MATLAB.

All data was spatially co-registered to the subjects' baseline T1W-Gd images in a four-step procedure using ElastiX.

Rigid body transformations were obtained between the DTI $S_0$ maps from both time points: baseline (week 0=$w_0$) and three weeks into this treatment ($w_3$) and the corresponding T1W-Gd images from the same time points. Rigid body transformations and non-linear B-splin were obtained between the T1W-Gd images from the two time points, as in the submitted manuscript IV in further detail. All the above transformations were applied in order, to the MD maps. Example registration is shown in Figure 8.

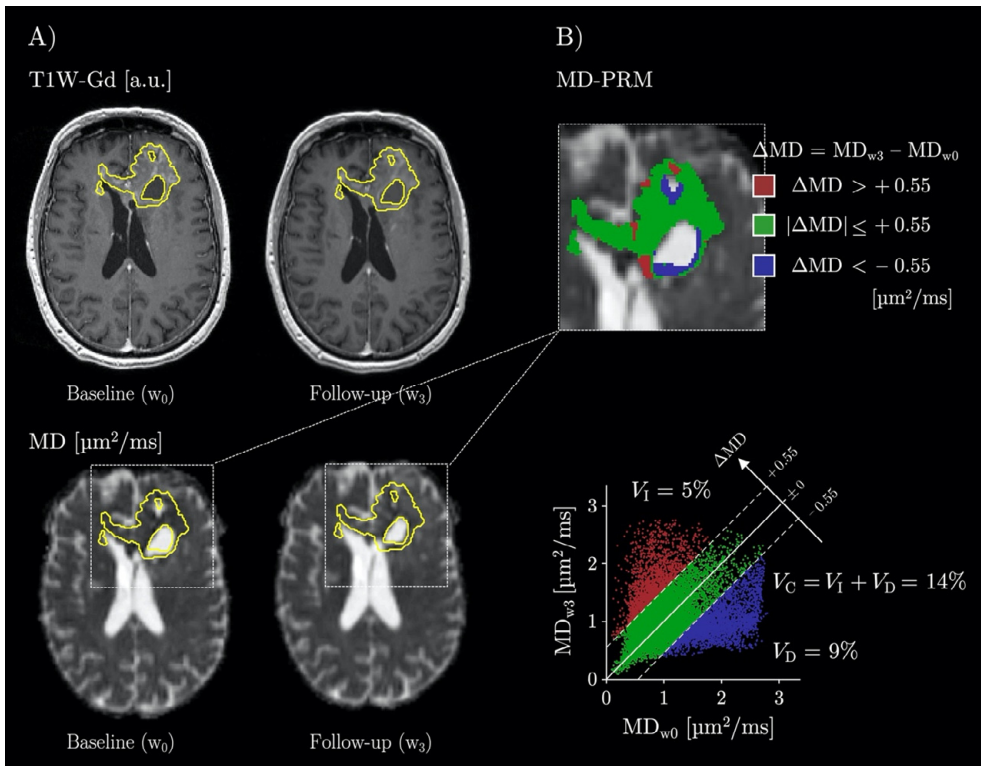


**Fig. 8 A-B. Demonstration of ROI definition in tumor tissue and the creation of PRM maps.**

**A.** ROI-definition in the co-registered image space. The tumors were manually defined at both time points in the T1W-Gd images (green lines). The intersection between the exam-specific ROIs (red lines) was then applied to the MD maps for the PRM analysis. **B.** MD-PRM maps were created from difference maps, ΔMD = MDw3 – MDw0, by classifying each voxel as increased (red), unchanged (green) or decreased (blue), using predefined thresholds. The percentage of increased, decreased, or changed (increased or decreased) define the MD-PRM metrics VI, VD and VC, respectively

*Creation of parametric response maps*

The tumors were defined at both time points: baseline ($w_0$) and week 3 ($w_3$) in the co-registered image space by manually placing regions of interest (ROIs) in the T1W-Gd images (Figure 8.], the delineation aimed to include all parts of the tumors that were enclosed by a ring of contrast enhancement expected viable tumor tissue but excluding the non-enhancing necrotic core and liquidized resection cavity. The final tumor ROIs used for analysis were obtained as the intersection between the two time points.

The change in MD between the two time points $w_0$ and $w_3$ was quantified voxel-by-voxel using the difference maps (ΔMD), which defined the parametric response maps (PRM) by classifying each voxel within the tumor ROI as either ”decreased” ($\Delta MD < -0.55\ \mu m^2/ms$), “increased” $\Delta MD > 0.55\ \mu m^2/ms$, or “unchanged” (Figure 8). The percentage of voxels with increased MD; $V_I$, and the percentage of voxels with decreased MD; $V_D$, and resulting total percentage of changed voxels; $V_C = V_I + V_D$, were calculated.

# Neuropsychological, quality of life and clinical medical assessment

Patients included in the study protocol for papers II-IV were assessed for cognitive performance by the CNS-vital signs (CNS-VS) [129]. CNS-VS is a standardized computerized cognitive test, which has been used in different patient groups such as MS, post head and neck trauma, meningioma and autoimmune diseases (more in detail described in the introduction, section *Cognitive evaluation in brain tumor patients*). The test evaluates nine cognitive function domains: composite memory, verbal memory, visual (spatial) memory, executive functioning, information processing speed, psychomotor speed, reaction time, complex attention and cognitive flexibility, quantified in standard scores (ss): >109 above average; 90-109 average; 80-89 low average; 70-79 low; < 70 very low. In paper II, the CNS-VS test scores were correlated to survival parameters; time to progression (TTP) and overall survival.

Survival parameters and demographic data were retrieved from patients’ medical files.

Neurological symptoms were assessed by the National Institute of Health Stroke Scale NIHSS [142] for papers II-IV as part of the study protocol at baseline, defined as start of postoperative radio-chemotherapy and at 3.5 and 12 months from baseline. The ECOG performance scale [143] was assessed at abovementioned three time points and medication with corticosteroids and/or antiepileptics was registered.

Quality of life was assessed by the self-reporting questionnaire of the European Organization for Research and Treatment of Cancer QLQ-C30 (EORTC QLQ-C30) [144]. Presence of psychological symptoms of depression or anxiety were examined according to the self-reporting Hospital Anxiety and Depression Scale (HADS). [145]

# Statistical analysis

## Paper I

Quantitative volumetric analysis was performed by dividing the operated patients into six groups of sextiles depending respectively on A: residual tumor volume in mL, B: extent of resection in %.

Data was analyzed into Kaplan Mayer curves and survival analysis by Cox regression performed in uni- and multivariate analysis for progression-free survival and overall survival, adjusting for age, Karnovsky performance status, presence of eloquent tumor location, and additionally, if reoperation was performed or not, at tumor progression in relation to overall survival. Patients were censored if they were still alive on the day the data was collected or patients were lost to follow-up.

Visual inspection was compared to quantitative volumetric analysis by comparing inter-rater agreement as degree of agreement between the two methods by cross-tabulation and estimation of kappa value ($\kappa$). Interpretation of the kappa value was done according to the method of McHugh ML, 1 indicating total agreement, whereas a kappa of 0 meant any observed agreement was attributed to chance.

The degree of intra-rater agreement was analyzed by paired sample t-test to calculate the standard deviation of the differences. Thereafter this was quantified using the approach by Bland and Altman, [146,147] calculating two descriptive statistics: the repeatability coefficient and the coefficient of variation.

The statistical analyses were performed using SPSS v.21 (IBM Corp., Armonk, NY, USA)

## Paper II

Semiparametric Cox regression analyses were conducted with censoring for patients still alive/free of progression on October 1, 2017. The proportional hazards assumption was tested and appeared reasonable for all cognitive domains. In the first step, analysis adjusted one variable at a time were performed, where $p<0.05$

was considered statistically significant. Significant variables were subsequently included in the final model.

Collinearity tests were performed in order to see if several parameters responded similarly, by standard techniques.

Intraindividual changes in cognitive domain scores were analyzed by parametric regression-based t-test. The model assumptions were tested by QQ-plotting and plotting of standardized residuals by predicted values. A two-sided p-value below 0.05 was considered statistically significant.

All analyses were carried out using SAS 9.4, Cary, NC, USA.

## Paper III

Longitudinal DTI findings from the predefined time points, as change from baseline at the specific time points, changes in FA, MD, RD and AD, were analyzed by a linear mixed-effects model and fitted to the respective parameter values from each brain region, given by

$$Y_{ij} = \beta_0 + \beta_{1i} + \psi_j + \varepsilon_{ij}.$$

The dependent variable $Y_{ij}$ was the ROI-average parameter value at time point $i$ for subject $j$. The fixed effects comprised the baseline average $\beta_0 = \langle Y_{0j} \rangle$ as well as the average differences from the baseline at each time point $\beta_{1i} = \langle Y_{ij} \rangle - \beta_0$, where $\langle \cdot \rangle$ averages across subjects. The random effects comprised the random intercepts $\psi_j$ for subject $j$ as well as the random errors $\varepsilon_{ij}$, assumed to be normally distributed with 0 mean and the standard deviations $\sigma_{subject}$ and $\sigma_{error}$, respectively. Thus, the five variables of interest were the average differences from the baseline at weeks 3 through 52, given by $\beta_{1i}$ for $i = 2 \ldots 6$ ($\beta_{10}$ trivially equals 0). Thereafter, the relative change (%) per time point was calculated. In the analysis, parameter and structure subjects contributing data from fewer than 4 time points were excluded, as were data points differing by 3 or more mean average deviations from the median.

The impact of biodose and age on the change in diffusion parameters was analyzed by Pearson correlation. The impact of gender was assessed by performing t-tests between the male and female subjects for each parameter, structure and time point respectively. In each analysis, results of $p < 0.01$ were considered significant.

Statistical analyses were performed by using MATLAB.

## Paper IV

Tumor progression evaluation parameters at eight months and at twelve months were compared with the average of each PRM-metric $V_I$, $V_D$ and $V_C$ by unpaired t-tests assuming equal variances between the subjects with stable disease (SD) versus subjects with progressive disease (PD). Two-sided $p<0.05$ was considered significant for all tests.

Receiver operating characteristics (ROC) curve analyses were performed to obtain the overall predictive values for SD at 8 months and at 12 months for each PRM-metric by the area under the curve (AUC). Log-rank tests were used to compare the overall survival (OS) and the progression-free survival (PFS) between patients stratified, based on the median value of each PRM-metric. Continuous variables are presented with mean and inter-patient standard deviation. All statistical analysis was performed using MATLAB.

# Ethical considerations

## Ethics, consents and permissions.

All studies are parts of a research project, which has been approved by the Ethical Committee with the following ethical approval numbers: 2011/598, 2011/814, 2012/188, 2014/368, 2016/957.

In addition, a separate approval was given for each study subject (LUBB 02-19) by the local biobank at the Department of Pathology upon an accepted application to the Regional Ethical Review Board, Lund University (#642/2008; updated # 2018/37) (paper IV).

Paper I: This is a retrospective study and acquisition of patient data from medical records have been approved by the regional data protection board (Kvalitetsregister, vårdinformationssystem och beredning (S-KVB) KVB000256-2017-06) and in ethical permits (see above). No patients underwent any extra procedures or tests, nor were their outcomes affected by the study. After acquisition of data from medical records, all patients received coded ID numbers and were de-identified.

Papers II, III and IV include the cohort of study patients who participated in the same prospective longitudinal MRI study, with ethical approval as described above. All patients had given informed consent prior to being included in the study.

Procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration (1964 [148], amended in 1975, 1983,) of the World Medical Association [149].

All patients received coded ID numbers and were de-identified for the MRI study procedure and cognitive testing and their evaluation.

Ethical considerations of this study are the increased number of performed MRI examinations and the extra cognitive examinations. These were time consuming for the patients and may have been connected with worries concerning the results of these extra examinations.

MRI findings related to regular clinical management were evaluated by a neuroradiologist at the Department of Imaging and Physiology, section of neuroradiology, Skåne university hospital, Lund as part of the clinical routine to rule out any signs of progression in patients diagnosed with brain tumor. The information

of the clinical part from each MRI examination was, as a rule, given to the patient within 1-2 weeks, by the referring physician, as part of clinical practice/routine. If signs of progression were seen, this was evaluated/discussed in the regular multidisciplinary conference and assessment of further treatment was done according to clinical routine.

# Results

## Paper I

Subjective visual volumetric evaluation had minimal inter-rater agreement for extent of resection (kappa value=0.38) and only modest inter-rater agreement for residual tumor volume (kappa value=0.54), compared to the quantitative radiologic volumetric assessment (QRA).

The intra-rater agreement by visual estimation measurements using the formula (width x length x height x 0.5) showed standard deviations in pre- and postoperative tumor volume of 10.6mL and 14.5 mL respectively. By comparison, quantitative volumetric assessment had standard deviations of 2.4mL preoperatively and 2.8mL postoperatively. This resulted in both better/lower repeatability coefficient and coefficient of variation in the quantitative volumetric measurements both pre- and postoperatively, thereby QRA was concluded as more reliable.

Since quantitative volumetric assessment was superior to subjective measurement and to using the cross diameter formula, both in reliability and reproducibility and had lower variation, it was used as final measurement for the study.

By quantitative volumetric assessment 17/70 (24%) of patients had 0 mL postoperative residual tumor volume (RTV) and 100% extent of resection (EOR) respectively.

Predictive cut-offs were identified by the quantitative volumetric assessment: RTV< 1.6 mL was significant prognostic at 18 months in the Cox regression model, both related to progression-free survival (PFS) (Figure 9.A) in multivariate analysis including age, performance status by KPS ($p$=0.003) and related to overall survival (OS) in the univariate ($p$=0.001) and multivariate analysis ($p$=0.012). EOR > 96% was a significant prognostic factor at 18 months regarding PFS ($p$=0.043) (Figure 9.B), but not concerning OS.

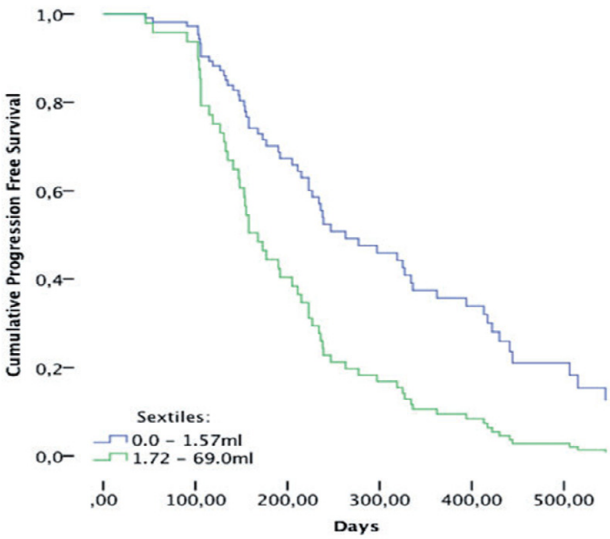


**Figure 9 A. PFS correlated to RTV. Cumulative PFS** in sextiles 1–3 vs. 4–6 from the Cox regression analysis of RTV, with right censoring at 548 days. Multivariate analysis of RTV adjusted for age, KPS, and tumor eloquence on PFS gave a HR of 0.44 (*P*=.0.003) between sextiles 1–3 (RTV<1.6 mL) vs. sextiles 4–6(RTV>1.6 mL). A significant prognostic benefit on PFS can be seen when achieving a RTV<1.6 mL.

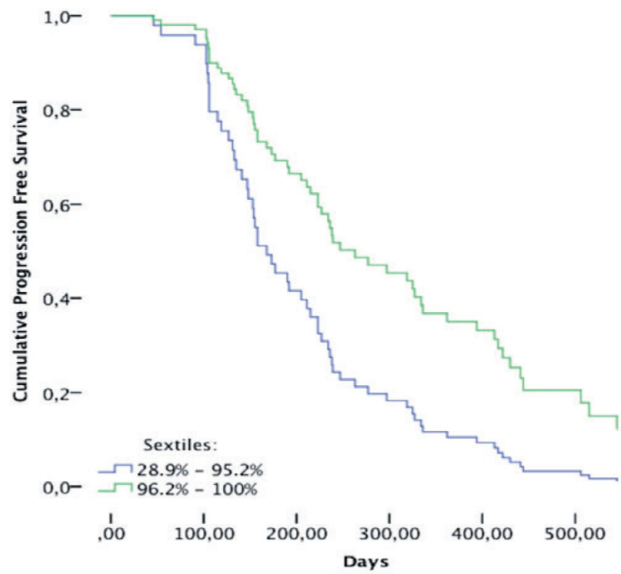


**Figure 9 B. PFS and its correlation to EOR. Cumulative PFS** sextiles 1–3 vs. 4–6 from the multivariate Cox regression analysis of EOR presented as percentages with right censoring at 548 days. Multivariate analysis of EOR adjusted for age, KPS, and tumor eloquence showed a significant survival benefit between sextiles 1–3 (EOR<96%) and sextiles 4–6 (EOR>96%) with a HR of 2.15 *(P*=0.005) on PFS indicating twice the chance of surviving with an EOR>96%.

RTV was superior in predicting survival in the favorably resected three sextiles (RTV< 1.6mL), compared to the three most extensively resected sextiles by EOR (EOR>96%): RTV was predictive for OS at both 1 and 1.5 years. This can be seen by statistical significance ($p$= 0.007, $p$=0.012) for RTV in the Cox regression model, including multivariate analysis. EOR had a weak prognostic significance on OS in the univariate cox-regression at 1.5 years ($p$=0.025), but not in the multivariate analysis neither at 1 year ($p$=0.052) nor at 1.5 years ($p$=0.053).

# Paper II

Out of 32 included patients, 31 performed cognitive testing by CNS-VS at baseline before start of radio-chemotherapy, 26/32 at 3.5 months and 13/32 at 12 months. Glioblastoma patients had median cognitive test scores in CNS-VS below average, in 7 out of 9 domains within "lower average" (Table 6). However, patients median test scores did not deteriorate significantly 3.5 or 12 months after treatment start by chemoradiotherapy, apart from the domain of Visual memory, where a deterioration was seen (Table 7).

**Table 6. Cognitive test score CNS-Vital Signs (CNS-VS) in Standard Score*** (ss) at baseline, follow up 3.5 months and 12 months.

| Cognitive test domaine standard scores (ss) | Baseline (N=31) | | | | Follow up 1 (3.5 months) (N=26) | | | | Follow up 2 (12 months) (N=13) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***MD*** | **Min** | **Max** | **SD** | ***MD*** | **Min** | **Max** | **SD** | ***MD*** | **Min** | **Max** | **SD** |
| **Cognitive flexibility domain** | 83.0 | 1 | 121 | 33.2 | 74.0 | 15 | 110 | 29.2 | 82.0 | 3 | 119 | 19.5 |
| **Complex attention domain** | 86.0 | 1 | 117 | 37.0 | 84.5 | -28 | 118 | 40.4 | 96.0 | -1 | 118 | 37.0 |
| **Executive functioning domain** | 80.0 | 1 | 120 | 32.5 | 65.5 | 2 | 110 | 30.9 | 84.0 | 9 | 119 | 34.5 |
| **Memory domain (verb+visual)** | 90.0 | 1 | 130 | 22.7 | 84.5 | 20 | 118 | 24.4 | 90.0 | 20 | 114 | 27.6 |
| **Processing speed domain** | 83.0 | 2 | 128 | 25.5 | 85.5 | 30 | 114 | 23.6 | 97.0 | 32 | 130 | 27.5 |
| **Psychomotor speed domain** | 100.0 | 7 | 132 | 29.8 | 95.0 | 33 | 121 | 24.0 | 94.0 | 31 | 126 | 25.6 |
| **Reaction time** | 81.0 | 1 | 111 | 32.1 | 78.0 | 13 | 110 | 31.9 | 85.0 | -21 | 110 | 42.5 |
| **Verbal memory_domain** | 87.0 | 1 | 122 | 28.3 | 87.5 | 1 | 119 | 30.4 | 97.0 | 12 | 121 | 32.0 |
| **Visual memory_domain** | 89.0 | 7 | 125 | 20.4 | 86.0 | 53 | 112 | 19.5 | 87.0 | 44 | 110 | 16.6 |

MD=median, SD= standard deviation.
*Test scores in standard scores (ss) >109 above average; 90-109 average; 80-89 low average; 70-79 low; < 70 very low.

**Table 7. Differences intra-individually in cognitive test results CNS-Vital Signs (CNS-VS)** in Standard Score (ss) in each tested cognitive domain at follow up 1 and at follow up 2 compared to baseline.

| Cognitive test domaine | Follow up 1 (3.5 months) - baseline (N=25)[a] | | | | Follow-up 2 (12 months) - baseline (N=13)[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | *MD* | Min | Max | SD | *MD* | Min | Max | SD |
| **Cognitive flexibility domain** | -2 | -76 | 59 | 26.8 | -2 | -84 | 25 | 30.3 |
| **Complex attention domain** | -2 | -74 | 48 | 29.0 | 0 | -98 | 62 | 42.2 |
| **Executive functioning domain** | -4 | -72 | 35 | 25.2 | -1 | -80 | 63 | 35.5 |
| **Memory domain(sum verbal+visual)** | -4 | -53 | 31 | 19.7 | 0 | -43 | 9 | 17.2 |
| **Processing speed domain** | -3 | -24 | 27 | 13.2 | 0 | -45 | 51 | 27.0 |
| **Psychomotor speed domain** | -5 | -42 | 54 | 19.2 | -7 | -68 | 71 | 34.2 |
| **Reaction time domain** | 1 | -49 | 36 | 21.3 | 0 | -57 | 59 | 29.9 |
| **Verbal memory_domain** | -3 | -90 | 39 | 30.2 | -3 | -60 | 15 | 19.3 |
| **Visual memory_domain** | -5 | -26 | 17 | 12.1 | -6 | -37 | 17 | 14.2 |

MD=median, SD= standard deviation. [a] 3.5 months test results minus baseline test results. [b] 1 year test results minus baseline test results.

Low test scores at treatment start for visual memory ($p$=0.0022) and executive function ($p$<0.0001) significantly correlated with shorter time to progression/ progression-free survival. Low executive function at baseline correlated most significantly ($p$=0.0013) with shorter overall survival.

Patients with non-frontal tumors performed significantly better in all four predominantly studied cognitive domains compared to patients with frontal tumors. In general, the test results in right sided tumor patients were better, compared to patients with left sided tumors.

Good (=normal) NIHSS scores were associated with better test scores in verbal memory ($p$=0.001) and executive function domains ($p$=0.03).

The patients self-reported their health and quality of life in EORTC QLQ-C30 as average to good and their psychiatric health as average according to the HADS self-assessment test results with no change at 3.5 or 12 months. There was no significant correlation between cognitive test results and quality of life parameters or anxiety and depression measurements.

# Paper III

DTI examinations from 27 glioblastoma patients were monitored 6 -12 months from initiation of chemo-radiotherapy. In the body of the corpus callosum, consistent significant changes, mainly by decreasing FA and increasing RD, especially at 15

weeks were observed. Otherwise, in irradiated normal-appearing brain tissue, only sporadicly, significant changes in diffusion parameters by DTI were observed during and up to twelve months after standard Stupp therapy, including arc-based rotation therapy (Table 8.) There was no correlation between radiation dose, age or gender and the diffusion parameters.

**Table 8. Changes in diffusion parameters per structure at each predefined timepoint** (w=weeks from baseline). The values in the week columns are the coefficients estimated in the mixed-model analysis. No corrections for multiple comparisons were applied.

| **FA [1]** | | | Change from baseline (BL) | | | | |
|---|---|---|---|---|---|---|---|
| Structure | Biodose [Gy] | BL | 3 w | 6 w | 15 w | 26 w | 52 w |
| CC Genu | 19 (15) | 0.76 (0.06) | 0.00 | -0.01 | -0.02** | -0.01 | -0.03**** |
| CC Body | 30 (19) | 0.64 (0.05) | -0.02** | -0.02** | -0.03*** | -0.02* | -0.02 |
| CC Splenium | 33 (20) | 0.79 (0.08) | -0.01 | 0.00 | -0.01 | 0.01 | 0.01 |
| CSO right | 22 (15) | 0.34 (0.05) | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 |
| HC left | 16 (19) | 0.15 (0.01) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| HC right | 17 (19) | 0.15 (0.01) | 0.00 | 0.01* | 0.01 | 0.01* | 0.00 |
| AM left | 16 (19) | 0.15 (0.02) | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 |
| AM right | 17 (19) | 0.15 (0.02) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **MD [$\mu m^2/ms$]** | | | Change from baseline (BL) | | | | |
| Structure | Biodose [Gy] | BL | 3 w | 6 w | 15 w | 26 w | 52 w |
| CC Genu | 19 (15) | 0.71 (0.06) | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 |
| CC Body | 30 (19) | 0.79 (0.05) | 0.02* | 0.03** | 0.03*** | 0.03*** | 0.03** |
| CC Splenium | 33 (20) | 0.66 (0.07) | 0.01 | -0.01 | -0.01 | -0.02 | -0.04** |
| CSO right | 23 (16) | 0.78 (0.06) | -0.01 | -0.01 | -0.01 | -0.02** | -0.01 |
| HC left | 18 (19) | 0.84 (0.02) | 0.00 | -0.01 | 0.00 | -0.01 | -0.01 |
| HC right | 19 (20) | 0.85 (0.04) | -0.01 | -0.01 | -0.01 | -0.02** | 0.00 |
| AM left | 16 (19) | 0.78 (0.04) | 0.00 | -0.01 | -0.01 | -0.02* | -0.01 |
| AM right | 16 (19) | 0.77 (0.04) | 0.00 | 0.00 | 0.00 | -0.01 | 0.00 |
| **RD [$\mu m^2/ms$]** | | | Change from baseline (BL) | | | | |
| Structure | Biodose [Gy] | BL | 3 w | 6 w | 15 w | 26 w | 52 w |
| CC Genu | 19 (15) | 0.31 (0.07) | 0.01 | 0.01 | 0.02** | 0.01 | 0.03*** |
| CC Body | 30 (19) | 0.45 (0.06) | 0.03*** | 0.03*** | 0.04**** | 0.04*** | 0.04** |
| CC Splenium | 33 (20) | 0.26 (0.10) | 0.01 | 0.00 | 0.01 | -0.01 | -0.02 |
| CSO right | 23 (16) | 0.64 (0.07) | -0.01 | -0.01 | -0.01 | -0.03** | -0.01 |
| HC left | 17 (19) | 0.78 (0.03) | 0.00 | -0.01 | 0.00 | -0.01 | -0.01 |
| HC right | 19 (20) | 0.79 (0.04) | -0.01 | -0.01 | -0.01 | -0.02** | 0.00 |
| AM left | 16 (19) | 0.72 (0.04) | 0.00 | -0.01 | -0.01 | -0.02* | -0.01 |
| AM right | 17 (19) | 0.70 (0.04) | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 |
| **AD [$\mu m^2/ms$]** | | | Change from baseline (BL) | | | | |
| Structure | Biodose [Gy] | BL | 3 w | 6 w | 15 w | 26 w | 52 w |
| CC Genu | 22 (17) | 1.51 (0.09) | -0.01 | -0.03 | 0.00 | -0.02 | -0.05* |
| CC Body | 30 (19) | 1.49 (0.08) | 0.01 | 0.01 | 0.02 | 0.03 | 0.03 |
| CC Splenium | 36 (21) | 1.44 (0.10) | 0.01 | -0.01 | -0.02 | -0.01 | -0.05* |
| CSO right | 22 (15) | 1.06 (0.06) | -0.01 | 0.00 | -0.01 | -0.01 | -0.01 |
| HC left | 18 (19) | 0.97 (0.03) | 0.00 | -0.01 | 0.00 | -0.01 | 0.00 |
| HC right | 18 (20) | 0.97 (0.04) | -0.01 | -0.01 | 0.00 | -0.02* | 0.00 |
| AM left | 16 (19) | 0.90 (0.05) | -0.01 | -0.01 | -0.01 | -0.02* | -0.01 |
| AM right | 16 (19) | 0.89 (0.04) | 0.00 | 0.00 | 0.00 | -0.02 | 0.00 |

CC, corpus callosum; CSO, centrum semiovale; HC, hippocampus; AM, amygdala. The CSO left was excluded from analysis due to tumor infiltration in a majority of cases. * = $p < 0.05$. ** = $p < 0.01$ .*** = $p < 0.001$. **** = $p < 0.0001$. Biodose and BL values are given as mean (inter-subject SD).

# Paper IV

MRI from 31 patients from baseline and 3 weeks into treatment were analyzed.

MD-PRM change at 3 weeks into chemo-radiotherapy was not predictive of stable disease or progressive disease at 8 or 12 months. Patients with an above median Mean Diffusivity (MD) reduction $V_D$ measured by PRM had a slightly longer PFS ($p$=0.015) in Kaplan-Maier analysis. OS was non-significantly longer ($p$=0.099).

However, in the sub-group of patients only undergoing biopsy, PRM-MD change at 3 weeks had high prognostic specificity, but low sensitivity for therapy response as SD or PD at 8 months by simple swarm plots: $V_I$ (1.0% vs 0%), $V_D$ (2% vs 0%) and $V_C$ (3% vs 0%). Due to small sample size, no t-test or other statistics were performed in this subgroup (Figure 9).

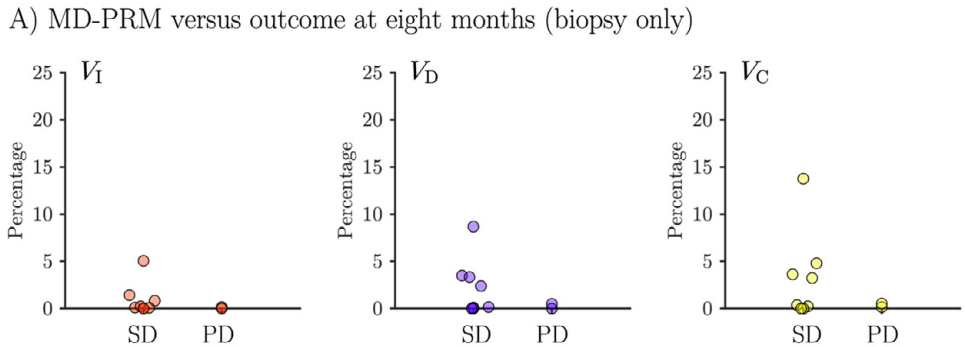


**Figure 9. MD-PRM metrics in patients undergoing biopsy only at 8 months**.Comparison of the MD-PRM-metrics $V_I$ (red), $V_D$ (blue) and $V_C$ (yellow) between patients who only underwent diagnostic biopsy (dots, $n$ = 10) with stable disease (SD) and progressive disease (PD) at eight months (A). Patients with SD appeared to feature higher values for all three metrics at eight months, although no statistical tests were performed.

MGMT status had a clear prognostic correlation with survival: PFS ($p < 0.01$, log-rank test) and OS ($p < 0.01$) being significantly longer in MGMT-positive patients compared to non-MGMT-methylated patients. MGMT-status did not correlate with MD-PRM change at 3 weeks.

# Discussion

## Brief summary of observations

This thesis focuses on optimal monitoring of glioblastoma patients by MR imaging and cognitive testing to predict prognosis and effects of therapy.

We have studied the importance of absolute and relative degree of surgical tumor resection in relation to progression-free and overall survival by quantitative measurement in comparison with visual estimation in glioblastoma patients (paper I). We sought to identify a prognostic threshold: especially residual tumor volume of <1,6 mL was shown to be prognostic for progression-free and overall survival. Furthermore, the quantitative volumetric method was by far superior in inter-rater and in intra-rater reproducibility compared to conventional estimation by a formula and pure visual evaluation.

We have longitudinally surveyed glioblastoma patients planned for radiotherapy with arc-based methods and standard chemotherapy, with focus on cognition and advanced MRI parameters.

Cognitive function was examined prior to, during and after radiotherapy and chemotherapy up to one year by a reproducible standardized method. Thereafter the correlated cognitive function was studied in relation to other clinical factors and the correlation of cognition at therapy initiation to progression-free and overall survival (paper II).

Within the same patient cohort of glioblastoma patients, the influence of radio-chemotherapy was surveyed longitudinally with advanced MRI including the study of microstructural signs by diffusion tensor imaging (DTI) up to one year (paper III). Specifically normal-appearing radiated brain structures were analyzed and the effects of radiation dose on tissue expected to be vulnerable for memory function including corpus callosum and structures of the limbic system.

A general observation made in both studies II and III was the lack of change over time, with almost total absence of cognitive deterioration and the minimal change in microstructure of analyzed structures by DTI up to one year after treatment begin with arc-based radiotherapy such as VMAT.

We further investigated the same patient cohort by parametric response map of intra-tumoral mean diffusivity MD (MD-PRM), aiming for early prediction of treatment

response (study IV). This was measured by analyzing the voxel-wise difference amount (V) in MD before start of therapy and three weeks into therapy, without detecting significant changes in correlation with therapy response at 8 or 12 months in the total cohort, thus not predictive. Minor signs postulating length of total progression-free survival by MD-PRM, were found among the patient group with higher amounts of voxels with decreased MD ($V_{D)}$., which had significantly longer PFS.

We did see a predictive value of MD-PRM on treatment response in the subgroup of patients who could only be operated by biopsy for diagnosis without tumor resection. Higher values of $V_I$, $V_D$ and $V_T$ were seen among biopsy patients with stable disease (SD), compared to biopsied patients with progressive disease (PD) at 8 months.

# Methodological considerations

There are some general methodological aspects to be mentioned:

### First (ongoing change of diagnostic classifications)

All studies were conducted with patients diagnosed with glioblastoma during the period from 2012 to 2019, which was defined as glioblastoma WHO grade IV according to the WHO classifications from 2007 and 2016, mainly based on the histology pattern. Patients were included and treated accordingly, before the latest WHO classification from 2021 and therefore without including IDH status for diagnosis, thus some patients with IDH mutation would now have a different diagnosis such as astrocytoma grade 4, with expected different prognosis and other molecular characteristics compared to glioblastoma grade 4/IV. This situation is shared with other longitudinal studies performed during that same period and has to be kept in mind, when comparing our results with future studies.

### Second (prognostic markers, radiological tumor definition)

In the retrospective MRI volumetric study I, MGMT-status was only accessible in a minority of patients, where MGMT-status may have an influence as a prognostic factor, in addition to performed multivariate analyses (including age and performance) on progression-free and overall survival. No patients only undergoing biopsy were included as comparison to degree of resection, which could have indicated a possible superiority in favor of biopsy compared to partial resection, or the contrary.

The study's final analysis was limited to grade of resected contrast enhanced (CE) tumor, the initial analysis of volumetry of non-contrast enhancing T2 and T2-FLAIR signaling changes, partly defined as vasogenic tumor oedema was rejected, as these findings were considered to have a clear potential of being influenced by the use of steroids. However, there is increasing knowledge indicating tumor spread in non-contrast enhanced areas. Recent findings by the RANO resect group suggest the importance of supramaximal resection beyond CE and that the volume of non-CE tumor areas above a specified threshold, have an impact on survival [150].

## Third (Drop-out, long term follow-up)

Studies II-III are both based on one-year longitudinal observations, where the study of longer observation periods would be desirable. But the severe prognosis of glioblastoma makes longer observation limited to few individuals with expected increasing proportion of dropouts. In our studies, only 40-50% of patients could be evaluated for follow up at twelve months, the results from the one-year survivors have therefore lower power. However, to this date few other studies have documented results of cognitive function or DTI parameters longitudinally more than 6-12 months from baseline of oncological therapy in glioblastoma patients.

With improved therapy combinations, the proportion of longer survivors are expected to increase and regimes to evaluate long-term effects on cognitive function and possible corresponding microstructural changes are warranted. However, this would require a substantial number of participating glioblastoma patients, which may not be achieved by a single center study. An interesting perspective in this context was presented at the annual meeting of the European association of neuro-oncology (EANO) in September 2022. EANO has recently formed an EANO guideline proposal *Diagnosis and management of complications from treatment of primary central nervous system tumors* [151](in press). In this proposal, the present variety in diagnosis and management of treatment associated adverse events was highlighted. The suggested intentions were to identify areas of interest and controversy, to generate pragmatic recommendations for prevention, recognition, management and follow-up of complications and to generate a "blueprint" for clinical trials in neuro-oncology.

## Forth (variation in/heterogenic type of surgery)

In study IV of diffusion parametric response maps MD-PRM (as in studies II-III) the cohort included a majority of patients, who had undergone gross total or partial tumor resection. Deformation of large resection cavities can make image co-registration challenging, by imperfect spatial alignment and transformation there is a higher risk of causing false MD changes due to tissue deformation. Additionally, as previously well-known and also observed in study I, the degree of tumor resection

and residual tumor volume influence progression-free survival with longer PFS in patients undergoing gross total resection, as a separate confounder on survival and progression, not solely dependent of the remaining tumor tissue properties studied by diffusion PRM.

The subgroup of patients with biopsy-only lacks these methodological and confounding influences. Notably, the biopsy-only group of this study was on the other hand too small for statistical testing.

# General discussion

Quantitative volumetric assessment of contrast enhanced tumor from T1 MRI of resected glioblastoma performed in the study I (paper I) was in line with previous and later studies, showing significant correlations between the residual CE tumor volume (RTV), degree of tumor resection (EOR) and overall survival [41]. Longest survival was, as expected, among patients with 0 mL RTV or 100% resected CE tumor emphasizing the importance of so-called gross total resection. The predictive impact on progression-free survival by low RTV and high extent of resection (EOR) was in line with other studies and here, interestingly, even more significantly correlated to progression-free survival compared to overall survival. For both these survival parameters, the absolute measurement of RTV had better accuracy in survival prediction than EOR, as observed 2014 on overall survival by Grabowsky et al, and on progression-free survival by Chaichana et al [75,152] and in additional later volumetric studies. Surgical thresholds/cut-off points favorable on overall survival for a significant treatment effect were observed by dichotomization for RTV < 1.6mL and for EOR > 96%. This is in line with previous reports [39,75]. Concerning progression-free survival, it is worth noting, that the radiological definition of tumor progression has undergone changes, adding the important aspect of pseudo-progression [153], making comparison challenging with earlier study observations of progression-free survival [106].

These and other results highlight, that quantitative volumetry should be an important tool to improve surgical decision-making in aiding the surgeon to optimize the operation planning and resection standards. Implementation of volumetry in the radiological routine evaluation is therefore important. The method we used had high reliability compared to conventional methods of for example the diameter product, although the method used here is time consuming. The use of automatized volumetric methods should be of great interest, but in the choice of method and its developments, the issues of tissue differentiation, such as non-tumorous reactions and perioperative effects, need to be taken into account.

It remains to be proven if biopsy or partial resection [154] is preferable, this was not analyzed in the present study. Other studies showed larger and more eloquently

located tumors among patients only undergoing biopsy, which makes survival comparison complicated.

When analyzing postoperative tumor volume, EOR is a relative entity, expressed in %, whereas RTV is an absolute entity of volume, expressed in mL/$cm^3$. The RTV value is independent of preoperative tumor volume and a low RTV may therefore, compensate in a patient with a large, late discovered tumor, with some positive effect on the prognostic outcome. The same does not apply for EOR. For example, a small preoperative tumor which has a relatively low EOR postoperatively, will despite this, still have a low RTV and therefore expected to have a prognostically more favorable outcome, compared to a patient with the same EOR grade but larger preoperative tumor volume.

There is also a need to improve the possibility to compare glioma surgery resection grade and outcome between different study centra accurately, for future clinical trials. Therefore, Karschina et al recently reviewed the field and suggested a standard evidence based EOR resection grade, also including documentation of RTV and addressing supramaximal resection of non-CE tumor from T2/FLAIR MRI [155]. Six EOR categories were defined: Supramaximal resection including non-CE tumor beyond CE tumor borders (from T2/FLAR); Complete resection of CE tumor 100%; Near total resection of ≥ 95% CE tumor + ≤1$cm^3$ (=mL) CE RTV; Subtotal resection of CE tumor ≥80% + CE ≤5$cm^3$ RTV; Partial resection of CE tumor 1-79% + CE RTV >5cm3 ; Biopsy of CE tumor without tumor reduction.

This scheme was further developed in 2022 by the formation of the RANO resect group [150] where the prognostic value of EOR from 7 different study centra, were evaluated, finally forming 4 resection categories for future studies:

- Class 1 **supramaximal CE resection** EOR 100% + ≥60% of non-CE tumor+≤5$cm^3$ RTV
- Class 2 **maximal CE resection**:

  2A. Complete 100% resection of CE tumor + ≤60% non-CE tumor ±≥5$cm^3$ RTV OR

  2B. Near total resection of ≥ 95% CE tumor ± ≤1cm3 CE RTV
- Class 3 **submaximal resection**:

  3A. Subtotal resection of CE tumor ≥80% + CE RTV≤5cm3 OR

  3.B Partial resection of CE tumor 1-79% + CE RTV >5cm3
- Class 4 **biopsy**, no reduction of tumor volume

The final analysis of the RANO resect study showed progression-free and overall survival benefits by increasing EOR. The largest benefit was seen for supramaximal CE resection, which was also true when only analyzing postoperative absolute RTV.

RTV additionally had statistical prognostic significance also in the multivariate analysis adjusted for age, performance status and MGMT-status. Similar findings were not reached for EOR in the multivariate analysis.

For supramaximal CE resection it was noted, that a cut off of minimum EOR of non-CE of 60% or ≤ 5 cm3 of RTV/ remaining non-CE tumor retrieved from T2/FLAIR was required to achieve prognostic benefits and there was no increase of reported postoperative neurological deficits in this category. In this most recent multi-center study patients only undergoing biopsy had the shortest progression-free survival.

The recent results of the RANO-resect study, in summary, further confirmed similar observations to our own observations (paper IV), that RTV had higher prognostic survival impact compared to EOR. RANO resect additionally demonstrated the survival benefit of removing non-CE tumor of at least 60% or less than 5 cm3 RTV non-CE tumor.

## Treatment effects

A major point of this thesis is to elucidate the structural and functional effects of radiotherapy as used today with arc-based therapy including VMAT. In paper II we could see that the glioblastoma patients had lower cognitive function standard scores, compared to test standard average, before start of postoperative oncological therapy. Specifically in terms of reduced verbal and visual memory, attention and executive functioning, the patients' results were lower than standard average. This is in accordance with earlier studies [26,31]. Additionally, as observed in earlier studies, lower cognitive function results at therapy begin correlated with shorter overall survival [27] and progression-free survival.

However, in the present study no clear further deterioration was seen in these cognitive domains after three and twelve months from start of radiotherapy by arc-based therapy and chemotherapy. There is very limited documentation on cognitive function over time in glioblastoma, but a recent study by Bodensohn et al in high grade glioma grade III and IV indicated similar findings [31] to ours, nonetheless, a more varied patient cohort in terms of histopathologic diagnosis and in follow-up examinations interval was included. In the present study of paper II, the patient cohort constituted entirely of glioblastoma grade IV patients, the intervals of testing were precisely predefined and therefore the lack of cognitive deterioration raises the question if the presently used therapy and especially radiotherapy by arc-based rotation therapy is less toxic to the patients' brain. This is further supported by the study observation that: the lower the cognitive test results at baseline, the shorter the progression-free and overall survival, indicating that a more aggressive tumor growth may constitute the main reason for cognitive impairment in glioblastoma patients, at least within the first twelve months from diagnosis. In a murine model

study, less toxic effects on neural stem cells from the subventicular zone in cells irradiated with VMAT were seen, compared to those exposed to 3D-CRT [156], as a hypothesis in favour of less neurotoxicity by arc-based RT.

Thorough literature related to the effects of arc-based RT including VMAT on brain tissue and cognition is, at this point, rare in primary brain tumor patients with glioblastoma. Whether later neurotoxic effects of arc-based RT may occur after the space of twelve months, should be of interest and importance to study.

Since the features of tumor influences on patient cognitive function are important for the autonomy of the patient, quality of life and, importantly, on how to provide adequate support and information to caregivers, early cognitive testing is of value [49]. Moreover, a chance to improve or stabilize cognitive function and coping by cognitive rehabilitation [157] should be important and needs to be addressed as part of postoperative therapy. The timing when to perform cognitive testing, that is most beneficial for the patient, is to be identified [158].

We can see advantages of using the test CNS-VS in this setting, both in clinical practice as a screening method and follow up, and in future research for several reasons:

CNS-VS covers the most important cognitive functions including visual and verbal memory, attention and executive function, as well as reaction time, psychomotor speed, processing speed and cognitive flexibility. It is standardized, repeatable without learning effects and has a limited time consumption of approximately 30-45 minutes, which is feasible for most glioblastoma patients, despite fatigue.

A limitation for the use of CNS-VS in clinical practice could be the license cost connected with each performed test, this may be put in relation to staff /professionals required by conventional more time-consuming testing.

The results from paper III, examining diffusivity changes in the normal-appearing non tumorous irradiated brain by DTI MRI longitudinally from baseline therapy start, during and after radiotherapy and standard chemotherapy up to twelve months, were in analogy with the cognitive function test results described in paper II: very limited changes were observed. Up to one year, no clear indications of the classically described early, delayed or late radiation damages were observed, except for the body of the corpus callosum, and only sporadic and transient changes in MD, FA, RD and AD were seen in normal-appearing irradiated tissue structures of hippocampus, amygdala and corpus callosum (splenium and genu). The DTI changes in the body of corpus callosum were significant by a decrease of FA during the first months, a later increase in MD and more consistent increase in RD indicating radiation damage of this specific structure.

Neither radiotherapy dose, nor age or sex of the patient had a significant impact on the DTI findings in this study.

Apart from changes in the body corpus callosum, the findings are in contradiction with earlier reports of more clear effects at some time point in DTI on radiated parts, generally of the corpus callosum [159], on other normal-appearing white matter (NAWM) structures [115,159,160] and on the hippocampus [118]. These previous studies showed clear significant impact at some point on DTI in a dose dependent manner, mainly by reduction of FA [159], or increase in MD [160], a pattern which we could not observe.

Our results using DTI in the study of paper III, indicate that radiotherapy by arc-based rotation therapy such as VMAT may be less harmful to the radiated brain than earlier used radiotherapy technics. This observation is supported by the cognitive test results from paper II of the same patient cohort, which also remained stable without further deterioration under the observation period of 12 months.

However, this remains to be proven, when feasible, also by longer follow up.

Longitudinal observations in brain tumor patients are demanding and time consuming but are of high importance, as they may allow increased understanding of mechanisms, higher reliability and more differentiated conclusions and correlations in relation to cause, such as tumor infiltration or radiation induced damage in crucial regions [31,64,161]. Apart from Hope et al [160], very few studies have continuously surveyed DTI findings during and after radiotherapy as done in paper III.

In a longitudinal setting, it could also be favorable to include the study of preoperative DTI findings in normal-appearing white matter (NAWM). In a study of pre-operative low- and high-grade brain tumor patients, DTI deviations in (NAWM) showed correlation to cognitive function and final postoperative diagnosis including IDH-status [30]. It seemed that an increasing influence on NAWM beyond the suspected tumor area, with decreased mean FA and AD compared to healthy controls, as a possible result of tumor activity not visualized by conventional MRI but assumed as occult tumor cell invasion or infiltration of white matter. In the same study, higher FA and lower MD and RD values of NAWM were associated with better cognitive performance including verbal memory and executive function. These described preoperative white matter DTI changes could be kept in mind when defining the tumor area and they could be valuable to consider in future volumetric assessment and in preoperative planning. Furthermore, if pre-existence of DTI changes beyond CE and oedematous tumor areas can be seen, these might be informative for the planning of the radiotherapy.

With intension to early identify treatment response by mean-diffusivity parametric response mapping (MD-PRM), the study of paper IV was conducted. The results 3 weeks into treatment were in total not predictive to treatment response, neither at 8 nor at 12 months from therapy start, which is in contrast to experiences from earlier studies [108-110,162]. This may again be related to the cohorts of patients, the present study only investigating a homogenous group of patients all diagnosed with

glioblastoma WHO grade IV, in contrast to the majority of previous studies of varying tumor type and malignancy degree. Secondly, the choice of postoperative therapy is different in the present study as discussed above, all receiving radiotherapy by arc-based radiotherapy such as VMAT and with the maximum doses of 2Gy/ fraction and standard regiment of 60Gy/30fractions with concomitant TMZ. VMAT, may, as discussed in the studies of cognitive function and of DTI longitudinally, have a different impact on tissue microstructure compared to previously used radiotherapy by 3D-CRT or IMRT. Previous studies have also used higher or varying maximal radiation doses, above 60Gy, all of these factors may influence tissue differently at an early stage, also in relation to the predictive value of treatment response.

In the subgroup of patients only undergoing diagnostic biopsy, contrary to the main study cohort of paper IV, the MD-PRM, metrics $V_I$, $V_D$ and $V_C$ in summary appeared to be predictive for stable disease (SD)/treatment response at 8 months. We conclude this to be an important finding, by suggesting that MD-PRM may constitute a tool for clinical decision-making in cases where no resection can be performed, e.g. due to the location or multifocality of the primary tumor or too poor performance status of the patient to tolerate extensive surgery. As the prognosis in biopsied patients tends to be less favorable and therefore the patients' quality of life is of extra high priority, MD-PRM as a potential tool for predicting treatment response could be of special value within this patient group. In addition, and if possible, it could be considered to offer alternative or experimental therapy in cases of MD-PRM predicting absence of treatment response to standard treatment.

A practical limitation to the use of PRM as a potential clinical tool as in this study, is the method of manual delineation being rather time consuming. The PRM method may be more robust and less sensitive to delineation obstacles and disturbance of partial volume effects and postoperative changes, when applied specifically in patients only undergoing biopsy. However further studies of MD-PRM with main focus on biopsied patients are necessary to verify these findings.

The study of paper IV confirmed previous observations of the strong prognostic value of MGMT-promoter methylation as indicator for better treatment response and prognosis in glioblastoma [13,74,163]. The MGMT-promoter methylation status did not correlate with the degree of MD-PRM change, therefore it could be interesting to evaluate, whether the higher $V_D$ values found in patients with longer PFS could suggest that MD-PRM may provide independent prognostic value, particularly in the subgroup of patients only undergoing biopsy.

# Clinical impact

I: Quantitative volumetric assessment may aid pre- and postoperative surgical planning and decision-making and the volumetric results have a prognostic influence on postoperative progression-free and overall survival of glioblastoma patients.

II: Cognitive testing prior to start of the oncological treatment may aid in the treatment planning. Both in low grade and in glioblastoma patients, further longitudinal monitoring is warranted and may be essential to provide adequate rehabilitation and psychosocial support, where the used test CNS-VS is feasible and has many advantages. Recently, CNS-VS has been introduced as a tool for cognitive evaluation in low-grade glioma patients in Lund.

III: Diffusion analysis by DTI of normal-appearing radiated tissue suggest that radiotherapy by arc-based rotation therapy such as VMAT used today may be less harmful to the radiated brain than earlier radiotherapy technics.

IV: MD-PRM may have impact on prognosis for further oncological treatment planning of patients only undergoing biopsy, as aid in the decision-making and level of treatment intensity.

# Future perspective

In the future it would be valuable to study quantitative volumetry additionally, including supramaximal resection and, in glioblastoma patients undergoing biopsy only, in relation to progression-free and overall survival, aiming for improved survival outcomes and decision-making.

It would be valuable to directly analyze and correlate advanced imaging methods, such as DTI and MD-PRM integrated with cognition findings by CVS-VS from the present study cohort. As example we did not see significant longitudinal change of diffusion by DTI in amygdala, however a newly published study has shown dose dependent volume reduction in the radiated amygdala in malignant glioma patients 9-15 months after radiotherapy and concomitant chemotherapy [164]. It could be valuable to analyze the influence of radiation dose to the amygdala, using diffusion parameters and to study these in correlation with cognitive and quality of life test-results, obtained in the presented cohort, with a future perspective to provide guidance for improving quality of life and to reduce psychological and cognitive side effects for the patients.

In addition, to study other new MRI techniques such as amide proton-weighted transfer techniques (APTw) for future investigation of brain tumor patients and follow up of glioblastoma patients could be interesting. Our imaging study site is currently monitoring glioblastoma patients under treatment with standard treatment and tumor treating fields (TT-fields) by advanced MRI including APTw imaging.

Amide proton transfer weighted imaging (APTw) is a novel MRI method in development, which is an alternative to perfusion MRI in detecting viable tumor tissue and which may have improved accuracy. Instead of adding Gd contrast such as in pMRI, in this chemical exchange saturation technique (CEST), the amide proton exchange located on mobile proteins and peptides is analyzed [165,166] and expressed in terms of a so-called proton transfer ratio (PTR), with signs of increased protein water signal in malignant tumors. Hereby APTw has the advantage of being independent of the use of metal-based contrast IV agent and, additionally, may have improved resolution. APTw may have implications both in regards of preoperative diagnostic tumor grading and in treatment evaluation, in addition to conventional pMRI [167]. Presently, APTw is still considered primarily a method limited to research setting. However, recent APTw imaging studies indicate the potential

clinical value for diagnosis [168] and in the differentiation between tumor progression [169] and treatment-related effects.

It could be valuable to further study MD-PRM in patients only undergoing biopsy. Specifically including the baseline preoperative images, analyzed in relation to pre-irradiation and three weeks into radiation with intention to both analyzing (a) tumor growth rate before chemo-radiotherapy to early identify rapid growing tumors [170] and (b) to early identify chemo- and radiotherapy response.

# Conclusion

I. Quantitative Volumetric Assessment had a higher reliability in estimating residual tumor volume (RTV) and extent of resection (EOR) compared to both subjective visual and objective volume estimation using a simple formula. Quantitative volumetrics showed prognostic significance in relation to progression-free and overall survival in operated glioblastoma patients with higher significance of RTV compared to EOR.

II. Arc-based radiotherapy generally did not significantly affect cognitive function longitudinally up to twelve months after therapy began. Low cognitive test scores, especially in executive function, at the start of postoperative therapy correlated with shorter time to progression and overall survival.

III. Arc-based RT had only a minor effect on diffusion parameters by DTI longitudinally up to 12 months after initiated therapy, indicating limited toxicity by arc-based RT in irradiated normal-appearing tissue adjacent to the brain tumor.

IV. MD-parametric response maps did not predict treatment response or progression at 8 or 12 months in newly diagnosed glioblastoma patients as a whole but may have a predictive value for treatment response in patients undergoing biopsy only. Findings using MD-parametric response maps are independent of patient MGMT-status.

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# References


1. Ostrom QT, Bauchet L, Davis FG, et al. The epidemiology of glioma in adults: a "state of the science" review. Neuro Oncol. 2014 Jul;16(7):896-913.
2. Rasmussen BK, Hansen S, Laursen RJ, et al. Epidemiology of glioma: clinical characteristics, symptoms, and predictors of glioma patients grade I-IV in the the Danish Neuro-Oncology Registry. J Neurooncol. 2017 Dec;135(3):571-579.
3. Bauchet L, Ostrom QT. Epidemiology and Molecular Epidemiology. Neurosurg Clin N Am. 2019 Jan;30(1):1-16.
4. Ostrom QT, Edelson J, Byun J, et al. Partitioned glioma heritability shows subtype-specific enrichment in immune cells. Neuro Oncol. 2021 Aug 2;23(8):1304-1314.
5. Amirian ES, Armstrong GN, Zhou R, et al. The Glioma International Case-Control Study: A Report From the Genetic Epidemiology of Glioma International Consortium. Am J Epidemiol. 2016 Jan 15;183(2):85-91.
6. Sadetzki S, Bruchim R, Oberman B, et al. Description of selected characteristics of familial glioma patients - results from the Gliogene Consortium. Eur J Cancer. 2013 Apr;49(6):1335-45.
7. Kleihues P, Burger PC, Scheithauer BW. The new WHO classification of brain tumours. Brain Pathol. 1993 Jul;3(3):255-68.
8. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta neuropathologica. 2016 Jun;131(6):803-20.
9. Louis DN, Perry A, Wesseling P, et al. The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. Neuro Oncol. 2021 Aug 2;23(8):1231-1251.
10. Ma Q, Long W, Xing C, et al. Cancer Stem Cells and Immunosuppressive Microenvironment in Glioma. Front Immunol. 2018;9:2924.
11. Kleihues P, Louis DN, Scheithauer BW, et al. The WHO classification of tumors of the nervous system. J Neuropathol Exp Neurol. 2002 Mar;61(3):215-25; discussion 226-9.
12. Li N, Zhang Y, Sidlauskas K, et al. Inhibition of GPR158 by microRNA-449a suppresses neural lineage of glioma stem/progenitor cells and correlates with higher glioma grades. Oncogene. 2018 Aug;37(31):4313-4333.
13. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. The New England journal of medicine. 2005 Mar 10;352(10):997-1003.

14. Tesileanu CMS, Dirven L, Wijnenga MMJ, et al. Survival of diffuse astrocytic glioma, IDH1/2 wildtype, with molecular features of glioblastoma, WHO grade IV: a confirmation of the cIMPACT-NOW criteria. Neuro Oncol. 2020 Apr 15;22(4):515-523.
15. Chen R, Smith-Cohn M, Cohen AL, et al. Glioma Subclassifications and Their Clinical Significance. Neurotherapeutics. 2017 Apr;14(2):284-297.
16. Reuss DE, Sahm F, Schrimpf D, et al. ATRX and IDH1-R132H immunohistochemistry with subsequent copy number analysis and IDH sequencing as a basis for an "integrated" diagnostic approach for adult astrocytoma, oligodendroglioma and glioblastoma. Acta neuropathologica. 2015 Jan;129(1):133-46.
17. Behling F, Schittenhelm J. Oncogenic BRAF Alterations and Their Role in Brain Tumors. Cancers (Basel). 2019 Jun 8;11(6).
18. Le Rhun E, Preusser M, Roth P, et al. Molecular targeted therapy of glioblastoma. Cancer Treat Rev. 2019 Nov;80:101896.
19. Ghiaseddin. Adult precision medicine: learning from the past to enhance the future. Neuro-Oncology Advances. 2021:1–11.
20. Bauchet L, Mathieu-Daude H, Fabbro-Peray P, et al. Oncological patterns of care and outcome for 952 patients with newly diagnosed glioblastoma in 2004. Neuro Oncol. 2010 Jul;12(7):725-35.
21. Chang SM, Parney IF, Huang W, et al. Patterns of care for adults with newly diagnosed malignant glioma. JAMA. 2005 Feb 2;293(5):557-64.
22. Thust SC, Heiland S, Falini A, et al. Glioma imaging in Europe: A survey of 220 centres and recommendations for best clinical practice. Eur Radiol. 2018 Aug;28(8):3306-3317.
23. Lasocki A, Anjari M, rs Kokurcan S, et al. Conventional MRI features of adult diffuse glioma molecular subtypes: a systematic review. Neuroradiology. 2021 Mar;63(3):353-362.
24. Cronqvist S. Angiography and cerebral blood flow in malignant glioma. Acta Radiol Ther Phys Biol. 1969 Feb-Apr;8(1-2):78-85.
25. Armstrong TS, Vera-Bolanos E, Acquaye AA, et al. The symptom burden of primary brain tumors: evidence for a core set of tumor- and treatment-related symptoms. Neuro Oncol. 2016 Feb;18(2):252-60.
26. Taphoorn MJB, Klein M. Review: Cognitive deficits in adult patients with brain tumours [Review Article]. Lancet Neurology. 2004 1/1/2004;3:159-168.
27. Johnson DR, Sawyer AM, Meyers CA, et al. Early measures of cognitive function predict survival in patients with newly diagnosed glioblastoma. Neuro-Oncology. 2012;14(6):808-816.
28. van Kessel E, Schuit E, Huenges Wajer IMC, et al. Added Value of Cognition in the Prediction of Survival in Low and High Grade Glioma. Front Neurol. 2021;12:773908.
29. Mrowczynski OD, Yang AL, Liao J, et al. The Potential of Glioblastoma Patient Symptoms to Diagnose and Predict Survival. Cureus. 2021 Jul;13(7):e16675.

30. Jutten K, Mainz V, Gauggel S, et al. Diffusion Tensor Imaging Reveals Microstructural Heterogeneity of Normal-Appearing White Matter and Related Cognitive Dysfunction in Glioma Patients. Front Oncol. 2019;9:536.
31. Bodensohn R, Corradini S, Ganswindt U, et al. A prospective study on neurocognitive effects after primary radiotherapy in high-grade glioma patients. International Journal of Clinical Oncology. 2016 08//;21(4):642.
32. Armstrong CL, Goldstein B, Shera D, et al. The predictive value of longitudinal neuropsychologic assessment in the early detection of brain tumor recurrence. Cancer. 2003;97(3):649-656.
33. Butterbrod E, Bruijn J, Braaksma MM, et al. Predicting disease progression in high-grade glioma with neuropsychological parameters: the value of personalized longitudinal assessment. J Neurooncol. 2019 Sep;144(3):511-518.
34. Sinha R, Stephenson JM, Price SJ. A systematic review of cognitive function in patients with glioblastoma undergoing surgery. Neurooncol Pract. 2020 Mar;7(2):131-142.
35. Jacob J, Durand T, Feuvret L, et al. Cognitive impairment and morphological changes after radiation therapy in brain tumors: A review. Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology. 2018 Aug;128(2):221-228.
36. Sundgren PC, Cao Y. Brain irradiation: effects on normal brain parenchyma and radiation injury. Neuroimaging Clin N Am. 2009 Nov;19(4):657-68.
37. Hadjipanayis CG, Widhalm G, Stummer W. What is the Surgical Benefit of Utilizing 5-Aminolevulinic Acid for Fluorescence-Guided Surgery of Malignant Gliomas? Neurosurgery. 2015 Nov;77(5):663-73.
38. Watts C, Price SJ, Santarius T. Current concepts in the surgical management of glioma patients. Clin Oncol (R Coll Radiol). 2014 Jul;26(7):385-94.
39. Sanai N, Polley MY, McDermott MW, et al. An extent of resection threshold for newly diagnosed glioblastomas. J Neurosurg. 2011 Jul;115(1):3-8.
40. Kreth FW, Thon N, Simon M, et al. Gross total but not incomplete resection of glioblastoma prolongs survival in the era of radiochemotherapy. Ann Oncol. 2013 Dec;24(12):3117-23.
41. D'Amico RS, Englander ZK, Canoll P, et al. Extent of Resection in Glioma-A Review of the Cutting Edge. World Neurosurg. 2017 Jul;103:538-549.
42. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. The New England journal of medicine. 2005 Mar 10;352(10):987-96.
43. Teoh M, Clark CH, Wood K, et al. Volumetric modulated arc therapy: a review of current literature and clinical use in practice. The British journal of radiology. 2011 Nov;84(1007):967-96.
44. Scaringi C, Agolli L, Minniti G. Technical Advances in Radiation Therapy for Brain Tumors. Anticancer Res. 2018 Nov;38(11):6041-6045.

45. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol. 2009 May;10(5):459-66.
46. Malmström A, Grønberg BH, Marosi C, et al. Temozolomide versus standard 6-week radiotherapy versus hypofractionated radiotherapy in patients older than 60 years with glioblastoma: the Nordic randomised, phase 3 trial. The Lancet Oncology. 2012;13(9):916-926.
47. Perry JR, Laperriere N, O'Callaghan CJ, et al. Short-Course Radiation plus Temozolomide in Elderly Patients with Glioblastoma. The New England journal of medicine. 2017 Mar 16;376(11):1027-1037.
48. Stupp R, Taillibert S, Kanner A, et al. Effect of Tumor-Treating Fields Plus Maintenance Temozolomide vs Maintenance Temozolomide Alone on Survival in Patients With Glioblastoma: A Randomized Clinical Trial. JAMA. 2017 Dec 19;318(23):2306-2316.
49. Roth P, Pace A, Le Rhun E, et al. Neurological and vascular complications of primary and secondary brain tumours: EANO-ESMO Clinical Practice Guidelines for prophylaxis, diagnosis, treatment and follow-up. Ann Oncol. 2021 Feb;32(2):171-182.
50. Ruda R, Trevisan E, Soffietti R. Epilepsy and brain tumors. Current opinion in oncology. 2010 Nov;22(6):611-20.
51. de Bruin ME, van der Meer PB, Dirven L, et al. Efficacy of antiepileptic drugs in glioma patients with epilepsy: a systematic review. Neurooncol Pract. 2021 Oct;8(5):501-517.
52. Vargo M, Henriksson R, Salander P. Rehabilitation of patients with glioma. Handb Clin Neurol. 2016;134:287-304.
53. Katz S, Ford AB, Moskowitz RW, et al. Studies of Illness in the Aged. The Index of Adl: A Standardized Measure of Biological and Psychosocial Function. JAMA. 1963 Sep 21;185:914-9.
54. Mlinac ME, Feng MC. Assessment of Activities of Daily Living, Self-Care, and Independence. Arch Clin Neuropsychol. 2016 Sep;31(6):506-16.
55. Ribeiro M, Benadjaoud MA, Moisy L, et al. Symptoms of Depression and Anxiety in Adults with High-Grade Glioma: A Literature Review and Findings in a Group of Patients before Chemoradiotherapy and One Year Later. Cancers (Basel). 2022 Oct 22;14(21).
56. Caruso R, GiuliaNanni M, Riba MB, et al. Depressive Spectrum Disorders in Cancer: Diagnostic Issues and Intervention. A Critical Review. Curr Psychiatry Rep. 2017 Jun;19(6):33.
57. Zetterling M, Elf K, Semnic R, et al. Time course of neurological deficits after surgery for primary brain tumours. Acta Neurochir (Wien). 2020 Dec;162(12):3005-3018.
58. Strand PS, Berntsen EM, Fyllingen EH, et al. Brain infarctions after glioma surgery: prevalence, radiological characteristics and risk factors. Acta Neurochir (Wien). 2021 Nov;163(11):3097-3108.

59. Habets EJJ, Kloet A, Walchenbach R, et al. Tumour and surgery effects on cognitive functioning in high-grade glioma patients. ACTA NEUROCHIRURGICA. 2014;156(8):1451-1459.
60. Sandberg-Wollheim M, Malmstrom P, Stromblad LG, et al. A randomized study of chemotherapy with procarbazine, vincristine, and lomustine with and without radiation therapy for astrocytoma grades 3 and/or 4. Cancer. 1991 Jul 1;68(1):22-9.
61. Bell EH, Zhang P, Shaw EG, et al. Comprehensive Genomic Analysis in NRG Oncology/RTOG 9802: A Phase III Trial of Radiation Versus Radiation Plus Procarbazine, Lomustine (CCNU), and Vincristine in High-Risk Low-Grade Glioma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2020 Oct 10;38(29):3407-3417.
62. Hopewell JW, Wright EA. The nature of latent cerebral irradiation damage and its modification by hypertension. The British journal of radiology. 1970 Mar;43(507):161-7.
63. Reinhold HS, Calvo W, Hopewell JW, et al. Development of blood vessel-related radiation damage in the fimbria of the central nervous system. Int J Radiat Oncol Biol Phys. 1990 Jan;18(1):37-42.
64. Lumniczky K, Szatmari T, Safrany G. Ionizing Radiation-Induced Immune and Inflammatory Reactions in the Brain. Front Immunol. 2017;8:517.
65. Sheline GE, Wara WM, Smith V. Therapeutic irradiation and brain injury. Int J Radiat Oncol Biol Phys. 1980 Sep;6(9):1215-28.
66. Mehta P, Fahlbusch FB, Rades D, et al. Are hypothalamic- pituitary (HP) axis deficiencies after whole brain radiotherapy (WBRT) of relevance for adult cancer patients? - a systematic review of the literature. BMC Cancer. 2019 Dec 12;19(1):1213.
67. Handisurya A, Rumpold T, Caucig-Lutgendorf C, et al. Are hypothyroidism and hypogonadism clinically relevant in patients with malignant gliomas? A longitudinal trial in patients with glioma. Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology. 2019 Jan;130:139-148.
68. Habets EJ, Taphoorn MJ, Nederend S, et al. Health-related quality of life and cognitive functioning in long-term anaplastic oligodendroglioma and oligoastrocytoma survivors. J Neurooncol. 2014 Jan;116(1):161-8.
69. Durand T, Bernier MO, Leger I, et al. Cognitive outcome after radiotherapy in brain tumor. Current opinion in oncology. 2015 Nov;27(6):510-5.
70. Kim JH, Brown SL, Jenrow KA, et al. Mechanisms of radiation-induced brain toxicity and implications for future clinical trials. J Neurooncol. 2008 May;87(3):279-86.
71. Gondi V, Hermann BP, Mehta MP, et al. Hippocampal dosimetry predicts neurocognitive function impairment after fractionated stereotactic radiotherapy for benign or low-grade adult brain tumors. Int J Radiat Oncol Biol Phys. 2013 Feb 1;85(2):348-54.
72. Krayenbuehl J, Di Martino M, Guckenberger M, et al. Improved plan quality with automated radiotherapy planning for whole brain with hippocampus sparing: a comparison to the RTOG 0933 trial. Radiation Oncology. 2017;12(1).

73. Hegi ME, Liu L, Herman JG, et al. Correlation of O6-methylguanine methyltransferase (MGMT) promoter methylation with clinical outcomes in glioblastoma and clinical strategies to modulate MGMT activity. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2008 Sep 1;26(25):4189-99.
74. Hegi ME, Stupp R. Withholding temozolomide in glioblastoma patients with unmethylated MGMT promoter-still a dilemma? Neuro Oncol. 2015 Nov;17(11):1425-7.
75. Grabowski MM, Recinos PF, Nowacki AS, et al. Residual tumor volume versus extent of resection: predictors of survival after surgery for glioblastoma. J Neurosurg. 2014 Nov;121(5):1115-23.
76. Corell A, Ferreyra Vega S, Hoefling N, et al. The clinical significance of the T2-FLAIR mismatch sign in grade II and III gliomas: a population-based study. BMC Cancer. 2020 May 20;20(1):450.
77. Shaw EG, Berkey B, Coons SW, et al. Recurrence following neurosurgeon-determined gross-total resection of adult supratentorial low-grade glioma: results of a prospective clinical trial. J Neurosurg. 2008 Nov;109(5):835-41.
78. Carstam L, Corell A, Smits A, et al. WHO Grade Loses Its Prognostic Value in Molecularly Defined Diffuse Lower-Grade Gliomas. Front Oncol. 2021;11:803975.
79. Moseley ME, Cohen Y, Kucharczyk J, et al. Diffusion-weighted MR imaging of anisotropic water diffusion in cat central nervous system. Radiology. 1990 Aug;176(2):439-45.
80. Le Bihan D, Johansen-Berg H. Diffusion MRI at 25: exploring brain tissue structure and function. Neuroimage. 2012 Jun;61(2):324-41.
81. Bammer R. Basic principles of diffusion-weighted imaging. European journal of radiology. 2003 Mar;45(3):169-84.
82. Zhang L, Min Z, Tang M, et al. The utility of diffusion MRI with quantitative ADC measurements for differentiating high-grade from low-grade cerebral gliomas: Evidence from a meta-analysis. J Neurol Sci. 2017 Feb 15;373:9-15.
83. Overcast WB, Davis KM, Ho CY, et al. Advanced imaging techniques for neuro-oncologic tumor diagnosis, with an emphasis on PET-MRI imaging of malignant brain tumors. Curr Oncol Rep. 2021 Feb 18;23(3):34.
84. Le Bihan D, Mangin JF, Poupon C, et al. Diffusion tensor imaging: concepts and applications. Journal of magnetic resonance imaging : JMRI. 2001 Apr;13(4):534-46.
85. Nilsson M, van Westen D, Stahlberg F, et al. The role of tissue microstructure and water exchange in biophysical modelling of diffusion in white matter. MAGMA. 2013 Aug;26(4):345-70.
86. Pierpaoli C, Jezzard P, Basser PJ, et al. Diffusion tensor MR imaging of the human brain. Radiology. 1996 Dec;201(3):637-48.
87. Basser PJ, Pierpaoli C. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. Journal of magnetic resonance Series B. 1996 Jun;111(3):209-19.

88. Voets NL, Pretorius P, Birch MD, et al. Diffusion tractography for awake craniotomy: accuracy and factors affecting specificity. J Neurooncol. 2021 Jul;153(3):547-557.
89. Nimsky C, Ganslandt O, Hastreiter P, et al. Preoperative and intraoperative diffusion tensor imaging-based fiber tracking in glioma surgery. Neurosurgery. 2007 Jul;61(1 Suppl):178-85; discussion 186.
90. Essig M, Nguyen TB, Shiroishi MS, et al. Perfusion MRI: the five most frequently asked clinical questions. AJR Am J Roentgenol. 2013 Sep;201(3):W495-510.
91. Nguyen TB, Cron GO, Mercier JF, et al. Preoperative prognostic value of dynamic contrast-enhanced MRI-derived contrast transfer coefficient and plasma volume in patients with cerebral gliomas. AJNR American journal of neuroradiology. 2015 Jan;36(1):63-9.
92. Vlieger EJ, Majoie CB, Leenstra S, et al. Functional magnetic resonance imaging for neurosurgical planning in neurooncology. Eur Radiol. 2004 Jul;14(7):1143-53.
93. Zhu H, Barker PB. MR spectroscopy and spectroscopic imaging of the brain. Methods Mol Biol. 2011;711:203-26.
94. Durmo F, Rydelius A, Cuellar Baena S, et al. Multivoxel (1)H-MR Spectroscopy Biometrics for Preoprerative Differentiation Between Brain Tumors. Tomography. 2018 Dec;4(4):172-181.
95. Weinberg BD, Kuruva M, Shim H, et al. Clinical Applications of Magnetic Resonance Spectroscopy in Brain Tumors: From Diagnosis to Treatment. Radiol Clin North Am. 2021 May;59(3):349-362.
96. Wang Q, Zhang J, Xu W, et al. Role of magnetic resonance spectroscopy to differentiate high-grade gliomas from metastases. Tumour Biol. 2017 Jun;39(6):1010428317710030.
97. Bumes E, Fellner C, Fellner FA, et al. Validation Study for Non-Invasive Prediction of IDH Mutation Status in Patients with Glioma Using In Vivo (1)H-Magnetic Resonance Spectroscopy and Machine Learning. Cancers (Basel). 2022 Jun 2;14(11).
98. Ozturk-Isik E, Cengiz S, Ozcan A, et al. Identification of IDH and TERTp mutation status using (1) H-MRS in 112 hemispheric diffuse gliomas. Journal of magnetic resonance imaging : JMRI. 2020 Jun;51(6):1799-1809.
99. Durmo F, Latt J, Rydelius A, et al. Brain Tumor Characterization Using Multibiometric Evaluation of MRI. Tomography. 2018 Mar;4(1):14-25.
100. Lundemann M, Munck Af Rosenschold P, Muhic A, et al. Feasibility of multi-parametric PET and MRI for prediction of tumour recurrence in patients with glioblastoma. Eur J Nucl Med Mol Imaging. 2019 Mar;46(3):603-613.
101. Fatterpekar GM, Galheigo D, Narayana A, et al. Treatment-related change versus tumor recurrence in high-grade gliomas: a diagnostic conundrum--use of dynamic susceptibility contrast-enhanced (DSC) perfusion MRI. AJR Am J Roentgenol. 2012 Jan;198(1):19-26.
102. Pauleit D, Floeth F, Hamacher K, et al. O-(2-[18F]fluoroethyl)-L-tyrosine PET combined with MRI improves the diagnostic assessment of cerebral gliomas. Brain. 2005 Mar;128(Pt 3):678-87.

103. Munck Af Rosenschold P, Engelholm S, Ohlhues L, et al. Photon and proton therapy planning comparison for malignant glioma based on CT, FDG-PET, DTI-MRI and fiber tracking. Acta oncologica (Stockholm, Sweden). 2011 Aug;50(6):777-83.
104. Macdonald DR, Cascino TL, Schold SC, Jr., et al. Response criteria for phase II studies of supratentorial malignant glioma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1990 Jul;8(7):1277-80.
105. Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010 Apr 10;28(11):1963-72.
106. Ellingson BM, Wen PY, Cloughesy TF. Modified Criteria for Radiographic Response Assessment in Glioblastoma Clinical Trials. Neurotherapeutics. 2017 Apr;14(2):307-320.
107. Lemasson B, Chenevert TL, Lawrence TS, et al. Impact of perfusion map analysis on early survival prediction accuracy in glioma patients. Transl Oncol. 2013 Dec 1;6(6):766-74.
108. Moffat BA, Chenevert TL, Lawrence TS, et al. Functional diffusion map: a noninvasive MRI biomarker for early stratification of clinical brain tumor response. Proc Natl Acad Sci U S A. 2005 Apr 12;102(15):5524-9.
109. Hamstra DA, Chenevert TL, Moffat BA, et al. Evaluation of the functional diffusion map as an early biomarker of time-to-progression and overall survival in high-grade glioma. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(46):16759-16764.
110. Hamstra DA, Galban CJ, Meyer CR, et al. Functional diffusion map as an early imaging biomarker for high-grade glioma: correlation with conventional radiologic response and overall survival. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2008 Jul 10;26(20):3387-94.
111. Tsuruda JS, Kortman KE, Bradley WG, et al. Radiation effects on cerebral white matter: MR evaluation. AJR Am J Roentgenol. 1987 Jul;149(1):165-71.
112. Curnes JT, Laster DW, Ball MR, et al. MRI of radiation injury to the brain. AJR Am J Roentgenol. 1986 Jul;147(1):119-24.
113. Sundgren PC, Dong Q, Gomez-Hassan D, et al. Diffusion tensor imaging of the brain: review of clinical applications. Neuroradiology. 2004 May;46(5):339-50.
114. Khong PL, Leung LH, Fung AS, et al. White matter anisotropy in post-treatment childhood cancer survivors: preliminary evidence of association with neurocognitive function. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2006 Feb 20;24(6):884-90.
115. Nagesh V, Tsien CI, Chenevert TL, et al. Radiation-induced changes in normal-appearing white matter in patients with cerebral tumors: a diffusion tensor imaging study. Int J Radiat Oncol Biol Phys. 2008 Mar 15;70(4):1002-10.
116. Chapman CH, Nagesh V, Sundgren PC, et al. Diffusion tensor imaging of normal-appearing white matter as biomarker for radiation-induced late delayed cognitive decline. Int J Radiat Oncol Biol Phys. 2012 Apr 1;82(5):2033-40.

117. Tringale KR, Nguyen T, Bahrami N, et al. Identifying early diffusion imaging biomarkers of regional white matter injury as indicators of executive function decline following brain radiotherapy: A prospective clinical trial in primary brain tumor patients. Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology. 2019 Mar;132:27-33.
118. Bian Y, Meng L, Peng J, et al. Effect of radiochemotherapy on the cognitive function and diffusion tensor and perfusion weighted imaging for high-grade gliomas: A prospective study. Sci Rep. 2019 Apr 12;9(1):5967.
119. Nagtegaal SHJ, David S, Philippens MEP, et al. Dose-dependent volume loss in subcortical deep grey matter structures after cranial radiotherapy. Clin Transl Radiat Oncol. 2021 Jan;26:35-41.
120. Taylor BV, Buckner JC, Cascino TL, et al. Effects of radiation and chemotherapy on cognitive function in patients with high-grade glioma. Journal of Clinical Oncology. 1998;16(6):2195-2201.
121. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". Journal of Psychiatric Research. 1975;12(3):189-198.
122. Meyers CA, Hess KR. Multifaceted end points in brain tumor clinical trials: cognitive deterioration precedes MRI progression. Neuro Oncol. 2003 Apr;5(2):89-95.
123. Wechsler. WAIS-R manual : Wechsler adult intelligence scale - revised. 1981.
124. Benedict RHB, Schretlen D, Groninger L, et al. Hopkins Verbal Learning Test – Revised: Normative Data and Analysis of Inter-Form and Test-Retest Reliability. The Clinical Neuropsychologist. 2010;12(1):43-55.
125. Ruff RM, Light RH, Parker SB, et al. Benton Controlled Oral Word Association Test: reliability and updated norms. Arch Clin Neuropsychol. 1996;11(4):329-38.
126. Lezak MD. Neuropsychological assessment. 3 ed. 1995.
127. Lezak MD. Neuropsychological assessment in behavioral toxicology--developing techniques and interpretative issues. Scand J Work Environ Health. 1984;10 Suppl 1:25-9.
128. Reitan RM. Validity of the Trail Making Test as an Indicator of Organic Brain Damage. Perceptual and Motor Skills. 2016;8(3):271-276.
129. Gualtieri CT, Johnson LG. Reliability and validity of a computerized neurocognitive test battery, CNS Vital Signs [Article]. Archives of Clinical Neuropsychology. 2006 10 / 01 /;21(7):623-643.
130. Littleton AC, Register-Mihalik JK, Guskiewicz KM. Test-Retest Reliability of a Computerized Concussion Test: CNS Vital Signs. Sports health. 2015 Sep-Oct;7(5):443-7.
131. Gualtieri CT, Johnson LG. A computerized test battery sensitive to mild and severe brain injury. Medscape journal of medicine. 2008 Apr 15;10(4):90.
132. Nystedt J, Mannfolk P, Jonsen A, et al. Functional connectivity changes in core resting state networks are associated with cognitive performance in systemic lupus erythematosus. J Comp Neurol. 2019 Aug 1;527(11):1837-1856.

133. Cannerfelt B, Nystedt J, Jonsen A, et al. White matter lesions and brain atrophy in systemic lupus erythematosus patients: correlation to cognitive dysfunction in a cohort of systemic lupus erythematosus patients using different definition models for neuropsychiatric systemic lupus erythematosus. Lupus. 2018 Jun;27(7):1140-1149.
134. Meskal I, Gehring K, van der Linden SD, et al. Cognitive improvement in meningioma patients after surgery: clinical relevance of computerized testing. J Neurooncol. 2015 Feb;121(3):617-25.
135. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumours of the central nervous system. Acta neuropathologica. 2007 Aug;114(2):97-109.
136. Sawaya R, Hammoud M, Schoppa D, et al. Neurosurgical outcomes in a modern series of 400 craniotomies for treatment of parenchymal tumors. Neurosurgery. 1998 May;42(5):1044-55; discussion 1055-6.
137. Lacroix M, Abi-Said D, Fourney DR, et al. A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. J Neurosurg. 2001 Aug;95(2):190-8.
138. Klein S, Staring M, Murphy K, et al. elastix: a toolbox for intensity-based medical image registration. IEEE Trans Med Imaging. 2010 Jan;29(1):196-205.
139. Shamonin DP, Bron EE, Lelieveldt BP, et al. Fast parallel image registration on CPU and GPU for diagnostic classification of Alzheimer's disease. Front Neuroinform. 2013;7:50.
140. Smith SM. Fast robust automated brain extraction. Hum Brain Mapp. 2002 Nov;17(3):143-55.
141. Rex DE, Shattuck DW, Woods RP, et al. A meta-algorithm for brain extraction in MRI. Neuroimage. 2004 Oct;23(2):625-37.
142. Goldstein LB, Samsa GP. Reliability of the National Institutes of Health Stroke Scale. Extension to non-neurologists in the context of a clinical trial. Stroke. 1997 Feb;28(2):307-10.
143. Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol. 1982 Dec;5(6):649-55.
144. Aaronson NK, Ahmedzai S, Bergman B, et al. The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. J Natl Cancer Inst. 1993 Mar 3;85(5):365-76.
145. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand. 1983 Jun;67(6):361-70.
146. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. International Journal of Nursing Studies. 2010;47(8):931-936.
147. Martin Bland J, Altman D. Statistical Methods for Assessing Agreement between Two Methods of Clinical Measurement. The Lancet. 1986;327(8476):307-310.
148. Gandevia B, Tovell A. Declaration of Helsinki. Med J Aust. 1964 Aug 22;2:320-1.
149. World Medical A. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013 Nov 27;310(20):2191-4.

150. Karschnia P, Young JS, Dono A, et al. Prognostic validation of a new classification system for extent of resection in glioblastoma: a report of the RANO resect group. Neuro Oncol. 2022 Aug 12.
151. Weller M, Le Rhun E, Van den Bent M, et al. Diagnosis and management of complications from the treatment of primary central nervous system tumors in adults. Neuro Oncol. 2023 Feb 27.
152. Chaichana KL, Jusue-Torres I, Navarro-Ramirez R, et al. Establishing percent resection and residual volume thresholds affecting survival and recurrence for patients with newly diagnosed intracranial glioblastoma. Neuro Oncol. 2014 Jan;16(1):113-22.
153. Ellingson BM, Chung C, Pope WB, et al. Pseudoprogression, radionecrosis, inflammation or true tumor progression? challenges associated with glioblastoma response assessment in an evolving therapeutic landscape. J Neurooncol. 2017 Sep;134(3):495-504.
154. Chaichana KL, Jusue-Torres I, Lemos AM, et al. The butterfly effect on glioblastoma: is volumetric extent of resection more effective than biopsy for these tumors? J Neurooncol. 2014 Dec;120(3):625-34.
155. Karschnia P, Vogelbaum MA, van den Bent M, et al. Evidence-based recommendations on categories for extent of resection in diffuse glioma. Eur J Cancer. 2021 May;149:23-33.
156. Phillips T, Martin L, Kornblum H, et al. SU-E-T-03: Justification and Feasibility of Neural Stem Cell Sparing in Whole Brain Irradiation Using VMAT. Medical physics. 2012 Jun;39(6Part9):3702-3703.
157. Zucchella C, Capone A, Codella V, et al. Cognitive rehabilitation for early post-surgery inpatients affected by primary brain tumor: a randomized, controlled trial. J Neurooncol. 2013 Aug;114(1):93-100.
158. Bergo E, Lombardi G, Pambuku A, et al. Cognitive Rehabilitation in Patients with Gliomas and Other Brain Tumors: State of the Art. Biomed Res Int. 2016;2016:3041824.
159. Connor M, Karunamuni R, McDonald C, et al. Regional susceptibility to dose-dependent white matter damage after brain radiotherapy. Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology. 2017 May;123(2):209-217.
160. Hope TR, Vardal J, Bjornerud A, et al. Serial diffusion tensor imaging for early detection of radiation-induced injuries to normal-appearing white matter in high-grade glioma patients. Journal of magnetic resonance imaging : JMRI. 2015 Feb;41(2):414-23.
161. Makale MT, McDonald CR, Hattangadi-Gluth JA, et al. Mechanisms of radiotherapy-associated cognitive disability in patients with brain tumours. Nat Rev Neurol. 2017 Jan;13(1):52-64.
162. Galban CJ, Chenevert TL, Meyer CR, et al. Prospective analysis of parametric response map-derived MRI biomarkers: identification of early and distinct glioma response patterns not predicted by standard radiographic assessment. Clin Cancer Res. 2011 Jul 15;17(14):4751-60.

163. McAleenan A, Kelly C, Spiga F, et al. Prognostic value of test(s) for O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation for predicting overall survival in people with glioblastoma treated with temozolomide. Cochrane Database Syst Rev. 2021 Mar 12;3:CD013316.
164. Huynh-Le MP, Karunamuni R, Moiseenko V, et al. Dose-dependent atrophy of the amygdala after radiotherapy. Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology. 2019 Jul;136:44-49.
165. Zhou J, Heo HY, Knutsson L, et al. APT-weighted MRI: Techniques, current neuro applications, and challenging issues. Journal of magnetic resonance imaging : JMRI. 2019 Aug;50(2):347-364.
166. Zhou J, Lal B, Wilson DA, et al. Amide proton transfer (APT) contrast for imaging of brain tumors. Magn Reson Med. 2003 Dec;50(6):1120-6.
167. Park KJ, Kim HS, Park JE, et al. Added value of amide proton transfer imaging to conventional and perfusion MR imaging for evaluating the treatment response of newly diagnosed glioblastoma. Eur Radiol. 2016 Dec;26(12):4390-4403.
168. Durmo F, Rydhog A, Testud F, et al. Assessment of Amide proton transfer weighted (APTw) MRI for pre-surgical prediction of final diagnosis in gliomas. PLoS One. 2020;15(12):e0244003.
169. Jiang S, Eberhart CG, Lim M, et al. Identifying Recurrent Malignant Glioma after Treatment Using Amide Proton Transfer-Weighted MR Imaging: A Validation Study with Image-Guided Stereotactic Biopsy. Clin Cancer Res. 2019 Jan 15;25(2):552-561.
170. Stensjoen AL, Solheim O, Kvistad KA, et al. Growth dynamics of untreated glioblastomas in vivo. Neuro Oncol. 2015 Oct;17(10):1402-11.