

Malignant glioma

– the impact of treatment,
sociodemographic factors and
metabolomics on survival

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To my family

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Abstract

Background

Gliomas constitute a large group of brain tumors. Glioblastoma, isocitrate dehydrogenase (IDH)-wildtype is the most common and most aggressive type of glioma. The initial treatment often includes surgery and postoperative radiotherapy and/or chemotherapy. Despite extensive treatment, the overall survival for patients with glioblastoma, IDH-wildtype is short, around 10 months in an unselected patient cohort. The prognosis is affected by several factors related to the patient (for example age, world health organization (WHO) performance status (PS) and comorbidities), the tumor (molecular biology/mutations) and the given treatment.

Aims

The aim of this thesis was to investigate prognostic and predictive clinical, sociodemographic and metabolic factors in glioma, especially glioblastoma, IDH-wildtype.

Methods

Survival was studied for all adult patients with glioma, WHO grade 4 in northern Sweden from 1995–2015. Tumor tissues from 244 patients undergoing surgery for glioblastoma, IDH-wildtype or astrocytoma, IDH-mutant, grade 4 from 2005–2015 were collected and stored in a biobank. Clinical data on prognostic factors and treatment were collected and analyzed for the patients with a tumor tissue sample in the biobank (the biobank cohort). Metabolite analyses were performed on tumor tissue samples through mass spectrometry-based methods and the metabolite data were evaluated in relation to overall survival and time to next intervention.

Survival in relation to sociodemographic factors was studied for patients with glioma, WHO grade 1–4 using the RISK North database. In RISK North, the National Quality Registry for Central Nervous System Tumors and sociodemographic registries were linked to study socioeconomic status, cohabitation status, travel time to the regional hospital and region of residence. Socioeconomic status was estimated through educational level.

Results

In Study I, an increased survival from 6.9–10.3 months was observed over time for all glioblastoma patients in northern Sweden diagnosed between 1995–2015. Clinical prognostic factors were studied in the biobank cohort. In the multivariable analysis, longer survival was associated with younger age at diagnosis, good WHO PS, extensive surgery, type of postoperative treatment and absence of metabolic disease/diabetes and inflammatory disease.

In Study II, an association was found between higher educational level and longer survival for patients with glioma, WHO grade 3–4. No differences in prognosis were observed when analyzing cohabitation status, travel time to the regional hospital or region of residence for glioma, WHO grade 1–4.

In Study III, different metabolites were associated with survival and time to next intervention. For survival, the analysis highlighted mainly metabolites related to amino acid, carbohydrate, and fatty acid metabolism, and for time to next intervention, amino acids and amino acid metabolites. Three predictive metabolic markers were found in the group treated with resective surgery and radiochemotherapy: indolelactate, 5,6-dihydrouracil and uridine 5'-diphospho-N-acetylglucosamine. Prognostic and predictive metabolites were merged into scores that were associated with survival or time to next intervention in multivariable analyses adjusted for other prognostic factors.

Conclusion

This thesis enhances the knowledge of the factors associated with survival and treatment response in glioma. The progress in treatment is studied in a clinical setting where earlier known prognostic factors are validated, and new data is presented on sociodemographic factors and on metabolic markers. Glioblastoma is a disease with, in many cases a short, expected survival and there is a substantial need for continued research with the main goal to improve the treatment for the patients.

Original papers

This thesis is based on the following papers:

- I. Eriksson M¹, Kahari J, Vestman A, Hallmans M, Johansson M, Bergenheim AT, Sandström M. Improved treatment of glioma, WHO grade 4 – changes in survival over two decades at a single regional Centre. *Acta Oncol.* 2019; 58(3):334–341.
- II. Söderlund M, Almqvist C, Sjöström O, Dahlin AM, Sjöström S, Hellquist BN, Melin B, Sandström M. The impact of socioeconomic status on glioma survival: a retrospective analysis. *Cancer Causes Control.* 2025; 36:577–586.
- III. Söderlund M, Wibom C, Brännström T, Bergenheim AT, Johansson M, Melin B, Björkblom B, Sandström M. Metabolic Signatures Offer Prognostic and Predictive Information in Glioblastoma. Submitted manuscript.

Other papers by the author not appended in this thesis:

- IV. Björkblom B, Wibom C, Eriksson M¹, Bergenheim AT, Sjöberg RL, Jonsson P, Brännström T, Antti H, Sandström M, Melin B. Distinct metabolic hallmarks of WHO classified adult glioma subtypes. *Neuro Oncol.* 2022; 24(9):1454–1468.

¹ Surname changed from Eriksson to Söderlund in 2022.

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Abbreviations

5-ALA	5-aminolevulinic acid
ANOVA	analysis of variance
ATRX	ATP-dependent helicase
BBB	blood brain barrier
CDKN	cyclin-dependent kinase inhibitor
CI	confidence interval
CNS	central nervous system
CT	computed tomography
DNA	deoxyribonucleic acid
ECOG	eastern cooperative oncology group
EGFR	epidermal growth factor receptor
FDG	fluorodeoxyglucose
GC-MS	gas chromatography-mass spectrometry
GD	the geography database
Gy	Gray
HR	hazard ratio
IDH	isocitrate dehydrogenase
ITT	intention to treat
LC-MS	liquid chromatography-mass spectrometry
LISA	the longitudinal integration database for health insurance
MGMT	O-6-methylguanine-DNA methyltransferase
MMR	mismatch repair
MRI	magnetic resonance imaging
OS	overall survival
PCV	procarbazine lomustine vincristine
PET	positron emission tomography
PFS	progression free survival
PS	performance status
SAM	s-adenosyl-L-methionine
SES	socioeconomic status
TERT	telomerase reverse transcriptase
TPR	the total population registry
TTFields	tumor treating fields
TTNI	time to next intervention
U-CAN	Uppsala/Umeå Comprehensive Cancer Consortium
UDP-Glc-Nac	uridine 5'-diphospho-N-acetylglucosamine
WHO	world health organization
WHO CNS5	5 th ed. of the WHO Classification of Tumors of the CNS

Sammanfattning på svenska

Hjärntumörer är en stor sjukdomsgrupp som innefattar både godartade och elakartade tumörer. Den här avhandlingen beskriver olika former av gliom och fördjupar sig i den mest elakartade, glioblastom. Gliom delas in i fyra grader (1–4) enligt Världshälsoorganisationen (WHO) vilket baseras på tumörens aggressivitet. Glioblastom har tumörgrad 4. Vi har undersökt hur överlevnaden har utvecklats över tid och hur den påverkas av faktorer som samsjuklighet, given behandling, utbildningsnivå och restid till sjukhus. Vidare har vi undersökt hur tumörens ämnesomsättning samverkar med svar på behandling och patientens överlevnad.

Överlevnaden förbättras över tid

I det första delarbetet undersöker vi hur överlevnaden i glioblastom har förändrats över tid i norra Sverige. Under studietiden har behandlingen utvecklats både avseende kirurgiska metoder och medicinsk behandling. Vi visar att överlevnaden stegvis förbättrats över studieperioden från 6.9 månader för den första delen av studieperioden (1995–1996) till 10.3 månader för den sista delen (2010–2015). Vi har också undersökt hur överlevnaden påverkas av behandlingen och visar att både typ av kirurgi och efterföljande behandling med strålbehandling och cytostatika är relaterade till överlevnad.

Låg utbildningsnivå är kopplad till sämre överlevnad vid höggradigt gliom

I det andra delarbetet har vi samkört olika register för att undersöka hur sociodemografiska faktorer påverkar prognosen vid gliom. Vi ser att patienter med gliom av grad 3–4 med en låg utbildningsnivå (upp till nio års skolgång) har en kortare överlevnad jämfört med patienter som har fullföljt gymnasiet eller studerat på universitet eller högskola. För de övriga faktorerna som vi har undersökt (restid till regionsjukhus, om man bor ensam eller tillsammans med en annan vuxen samt vid vilken region i Sverige patienten har behandlats) hittar vi ingen koppling till överlevnaden.

Analys av tumörens ämnesomsättning bidrar med kunskap om överlevnad och behandlingssvar

I det tredje delarbetet undersöker vi tumörens ämnesomsättning hos grad 4 gliom och letar ämnen (metaboliter) som eventuellt kan användas som markörer för att få information om patientens förväntade överlevnad och/eller svar på behandling. Vi finner att olika metaboliter kan kopplas till överlevnad och behandlingssvar och ser att ämnesomsättningen skiljer sig åt mellan tumörer som uppvisar olika egenskaper (till exempel storlek och förändringar av gener). Vi visar också att vi genom att slå samman informationen från de individuella metaboliterna kan skapa ett sammanvägt index med information om förväntad överlevnad och behandlingssvar.

Sammanfattningsvis ger den här avhandlingen ökad kunskap om gliom och bidrar med en pusselbit till förståelsen för vad som påverkar överlevnad och svar på behandling. Tyvärr är glioblastom en allvarlig sjukdom där överlevnaden i många fall är kort, ofta kring eller under ett år. Därför behövs fortsatt forskning i området med det huvudsakliga målet att förbättra behandlingen och omhändertagandet för patienter med hjärntumörer.

Background

Glioma, World Health Organization (WHO) grade 4 is a severe disease with a short, expected survival. The tumors are located in the central nervous system (CNS), most often in the brain that is a highly important organ, and the disease commonly affects the patient's cognition which might impair communication and decision-making.

Despite the progress made in treatment of other cancer diseases, we have not yet seen a huge breakthrough in the treatment of glioma, WHO grade 4. There is a great need for better treatments to improve survival and there are several challenges to overcome to succeed. Due to the location of the tumor in the brain, extensive surgery is associated with a large risk of neurological deficits, and the infiltrative growth pattern of a glioma, WHO grade 4 makes the tumor impossible to fully remove surgically. The brain is, due to its location in the cranium highly protected from damage, and the blood brain barrier (BBB) impairs the access of drugs, at least to the non-contrast enhancing parts of the tumor.

To achieve progress in treatment of glioma, WHO grade 4, we need to better understand the disease. New, more effective treatments are crucial, but we also need an increased knowledge on how to use the available treatments in the best way. To approach this goal, we have to increase our knowledge about which factors that impact survival, how treatments work in daily clinical routine, and if other factors than treatments affect both care and prognosis.

This thesis aims at taking a step forward in understanding glioma and focuses on deepening our knowledge on prognostic clinical and sociodemographic factors and the role of metabolic markers in prognostication and the choice of treatment.

Glioma

Brain tumors are a large group of tumors including many diseases with different characteristics. Brain tumors are categorized according to the WHO Classification of Tumors of the CNS. The current fifth edition (WHO CNS5) was published in 2021. In WHO CNS5, the tumors are divided in twelve main groups with several subtypes. The main groups

and the subtypes for gliomas, glioneuronal tumors and neuronal tumors are listed in Table 1.

Gliomas are a heterogenous group of diseases, constituting the second largest group of primary brain tumors accounting for approximately 22%¹. The largest group is meningiomas that most often are benign tumors. Gliomas emanate from the supportive cells in the CNS, the glial cells, most commonly astrocytes, oligodendrocytes and ependymal cells².

Gliomas are subdivided according to malignancy grade from WHO 1 to 4 based on histology and molecular parameters. Grade 1 tumors generally have low proliferative potential and are often cured by surgical resection. Grade 2 tumors are infiltrative but with low proliferation rate and patients often live 10–15 years with the diagnosis. Grade 3 tumors show histological signs of malignancy such as nuclear atypia and enhanced mitotic activity and finally, grade 4 tumors are highly malignant with active mitosis, necrosis and a distinct infiltrative growth pattern^{2,3}.

Table 1 Main groups of the WHO CNS5 classification. The six subtypes of the main group gliomas, glioneuronal and neuronal tumors are shown in the right panel.

WHO CNS5 main groups	Subtypes
Gliomas, glioneuronal tumors, and neuronal tumors	Adult-type diffuse gliomas
	Pediatric-type diffuse low-grade gliomas
	Pediatric-type diffuse high-grade gliomas
	Circumscribed astrocytic gliomas
	Glioneuronal and neuronal tumors
	Ependymal tumors
Choroid plexus tumors	
Embryonal tumors	
Pineal tumors	
Cranial and paraspinal nerve tumors	
Meningioma	
Mesenchymal, non-meningothelial tumors involving the CNS	
Melanocytic tumors	
Haematolymphoid tumors involving the CNS	
Germ cell tumors	
Tumors of the sellar region	
Metastases to the CNS	

In this thesis, malignant glioma is defined as glioma, WHO grade 2–4, and the focus is on the adult-type diffuse gliomas with emphasis on glioblastoma, isocitrate dehydrogenase (IDH)-wildtype and astrocytoma, IDH-mutant, grade 4. Glioma, WHO grade 1–3 is included in the study cohort in Study II but not in the other studies.

During the study period covered by this thesis the tumors have been classified according to five different updates of the classification (1st–5th). In WHO CNS5 there is a more comprehensive change compared to the earlier versions since the classification is based not only on histologic appearance and immunohistochemistry but also on molecular pathologic analyses to achieve an integrated diagnosis ². In WHO CNS5, tumor grade is indicated with Arabic instead of Roman numerals. The updated classifications bring differences in diagnostic criteria over time and, most obvious, a change in the definition of glioblastoma. In Studies I and II, before the tumors were routinely analysed for IDH mutations, glioblastoma includes both IDH-wildtype and IDH-mutant tumors. In Study III, where the tumors were reclassified according to the revised 4th edition of the WHO classification from 2016, glioblastoma is subdivided by IDH status into IDH-wildtype and IDH-mutant. These changes in the classification complicates research since the diagnostic entities have changed slightly over time which makes comparisons between different time periods difficult to interpret. In this thesis, the WHO CNS5 nomenclature is used, and glioblastoma indicates an IDH-wildtype tumor. When both IDH-wildtype and IDH-mutant tumors are intended, glioma, WHO grade 4 is used.

Epidemiology

The incidence of glioma, WHO grade 4 varies in different parts of the world. In North America the annual incidence is approximately 3–4 cases per 100 000 ¹. In Sweden, the National Board of Health and Welfare report the combined incidence of glioma, WHO grade 3 and 4. For persons above 20 years of age, the incidence of glioma, WHO grade 3 and 4 in Sweden from 2015 to 2024 was 8.21 (range 7.71–9.48) for men, and 5.25 (range 4.42–5.87) for women respectively per 100 000 (age adjusted according to the population 2024) ⁴.

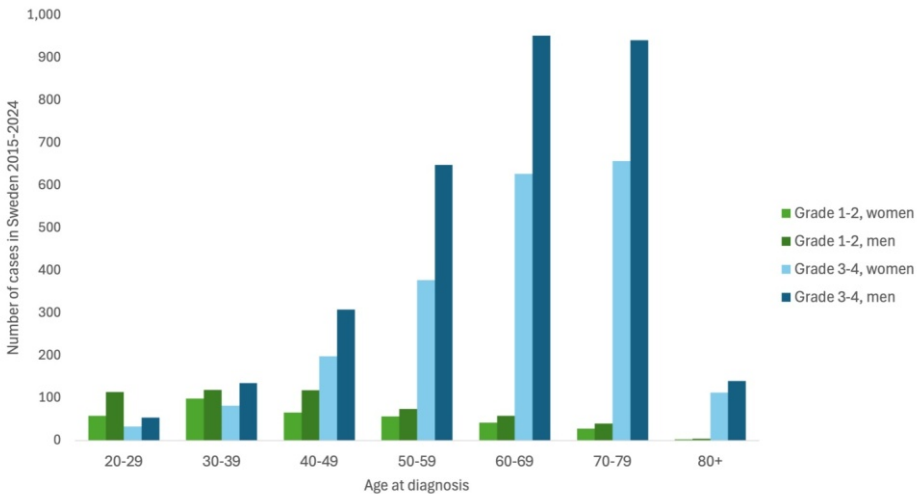


Figure 1 Number of cases of glioma, WHO grade 1–4 in Sweden 2015–2024 in different age groups. Data from The National Board of Health and Welfare, Statistical Database, Cancer ⁴.

The National Board of Health and Welfare reports in total 546 cases of glioma, WHO grade 3–4 for persons aged above 18 years in 2024 ⁴. Glioma, WHO grade 1–2 is less common than glioma, WHO grade 3–4 but more often affects younger persons (Figure 1). In data from the National Quality Registry for CNS Tumors, where all grades are reported separately, 486 adult persons were diagnosed with a glioma, WHO grade 4 and 35 persons with a glioma, WHO grade 3 in Sweden during 2023 ⁵. The coverage of the quality registry 2023 was $\geq 97\%$ in all regions ⁵.

Glioma, WHO grade 4 is more common among men than women with, in the Swedish context, 60% males and 40% females which gives a ratio of 1.55:1 ⁵. For glioma, WHO grade 4, the median age at diagnosis is 66 years ⁵.

Etiology

The etiology of glioma is to a large extent unknown and is most likely multifactorial with a combination of environmental, molecular and genetic factors.

The only known environmental risk factor for developing a glioma is earlier ionizing radiation against the head, for example treatment with radiotherapy during childhood ¹. The risk of developing a glioma in several previous studies found to be reduced by allergy and atopic disease ^{1,6-8}.

Some rare hereditary syndromes are associated with an increased risk of glioma. Examples of syndromes are Neurofibromatosis (NF) type 1 and 2 with aberrations in the genes *NF1* and *NF2* respectively, Li-Fraumeni syndrome with a germline mutation in the tumor suppressor gene *TP53*, constitutional mismatch repair deficiency syndrome with a defect in mismatch repair (MMR) genes, and melanoma-astrocytoma syndrome with pathogenic variants in the tumor suppressor gene cyclin-dependent kinase inhibitor (*CDKN2A*)². The known hereditary syndromes with an increased risk for glioma do not explain all cases where there is a suspected genetic association. Having a first-degree family member with a brain tumor is associated with a doubled risk of developing a primary brain tumor¹.

Prognosis

The median overall survival (OS) for an unselected cohort of glioma, WHO grade 4 patients is approximately 8–12 months^{1,9,10}. The two-year survival for glioma, WHO grade 4 after the introduction of postoperative radiochemotherapy in 2005 was 18% and the five-year survival for the same period was 4% in a meta-analysis where data from Study I were included¹¹. In Sweden, survival for patients with glioma, WHO grade 3–4 has improved over time, for example, an increased median OS from 8.1 months (95% confidence interval (CI) 7.3–8.8) between 1999–2003 to 10.0 months (95% CI 8.9–10.9) between 2004–2006¹² and in more recent data from the National Quality Registry for CNS tumor, the median OS for glioblastoma patients was 11.3 months between 2018–2021¹³.

Clinical presentation

The symptoms of a brain tumor depend on where in the brain the tumor is located and the size of the tumor. The tumor might also cause a surrounding edema enhancing the symptoms. Common symptoms are neurological deficits, seizures, cognitive or behavioral changes, and symptoms related to an increase in intracranial pressure such as headache, visual disturbances, nausea, and vomiting.

Diagnosis of glioma

When there is a clinical suspicion of a brain tumor, a neuroradiological examination should be performed. The initial examination is often a contrast enhanced computed tomography (CT) and thereafter, a contrast enhanced magnetic resonance imaging (MRI) scan to further characterize the tumor. In addition to CT and MRI, a positron emission tomography (PET) with an amino-acid trace labeled with ¹⁸F can be used

in selected cases, often to decide where in the tumor a biopsy should be taken.

In Sweden, a patient with a suspected cancer should be investigated according to a Cancer Patient Pathway. For brain tumors the Cancer Patient Pathway was introduced in 2015. The Cancer Patient Pathway for brain tumors starts with a well-founded suspicion of a brain tumor and specifies both which investigations that should be performed and which specific lead times to follow. For brain tumors, the well-founded suspicion can be a first-time epileptic seizure, focal neurologic symptoms, a new or different headache, or a suspected brain tumor on radiology performed on another indication ¹⁴.

Since there are many types of brain tumors, it is of utmost importance to achieve a tumor tissue sample for pathological diagnosis. The tumor tissue sample can be achieved from a biopsy of the tumor, or through a tumor resection. The tissue sample is analyzed through histopathology, and molecular pathological analyses to achieve a diagnosis and for treatment decisions.

Some brain tumors are not available for resection or, not even, biopsy depending on the location in the brain or patient- factors, e.g. comorbidities, WHO performance status (PS), or risks associated with anesthesia. In those cases, the most probable diagnosis is assessed from the radiology.

Molecular pathological analyses

In WHO CNS5, molecular pathological analyses are part of the classification of diffuse astrocytic or oligodendroglial gliomas. Prior to treatment decisions, especially in glioma, WHO grade 4, analysis of O-6-methylguanine-deoxyribonucleic acid (DNA) methyltransferase (*MGMT*)-promoter methylation is performed. These analyses are described in the following section and highlighted in Table 2 and Figure 2. In the last part of the section, the classification of glioblastoma, IDH-wildtype is described.

Mutations in *IDH1* and *IDH2* are analyzed for all astrocytomas and oligodendrogliomas to distinguish IDH-mutant from IDH-wildtype tumors ^{15,16}. The IDH genes encode for enzymes that catalyze the conversion of isocitrate to alpha-ketoglutarate in the citric acid cycle. In case of an IDH mutation, the enzyme loses its normal function, has a reduced affinity for isocitrate and instead produces 2-hydroxyglutarate

from alpha-ketoglutarate ¹⁷. The overproduction of 2-hydroxyglutarate has effects on gene regulation, epigenetic processes and tumor progression ^{2,18-20}. Lower grade gliomas are most commonly IDH-mutant, but in WHO grade 4 tumors only approximately 5% have an IDH mutation ^{7,17}.

The adenosine triphosphate (ATP)-dependent helicase (*ATRX*) gene encodes for a chromatin-binding protein involved in telomere function and DNA repair. Mutations in *ATRX* induce an abnormal telomere regulation and genomic instability and are associated with cell proliferation and longevity ^{15,21}. The presence of *ATRX* mutations indicate an astrocytic tumor in IDH-mutant diffuse gliomas ^{15,16}.

Deletion of the chromosomal arms 1p and 19q, described as 1p/19q codeletion, results in inactivation of tumor suppressor genes located on 1p or 19q ¹⁵. The combination of 1p/19q codeletion and IDH mutation defines oligodendroglioma ².

Table 2 Molecular pathological analyses recommended in the diagnosis of diffuse astrocytic or oligodendroglial gliomas.

Analysis	Genetic alteration	Analysis recommended for
IDH mutation	IDH1-R132H, IDH1-R132C, IDH2-R172K and IDH2-R172M	Astrocytoma and oligodendroglioma ^{15,16}
<i>ATRX</i> mutation	Inactivating <i>ATRX</i> mutations	Diffuse glioma, IDH-mutant if 1p/19q-codeletion is not excluded ¹⁶
1p/19q codeletion	Deletion of 1p and 19q	Diffuse glioma, IDH-mutant and without loss of <i>ATRX</i> ^{15,16}
<i>CDKN2A/CDKN2B</i>	Often deletions	Astrocytoma, IDH-mutant ¹⁵
Chromosome 7/10 alteration	Chromosome 7 gain and chromosome 10 loss	Astrocytoma, IDH-wildtype, grade 2–3 ¹⁶
<i>TERT</i> promoter mutation	Mutations in <i>TERT</i> promoter	Diffuse glioma, IDH-wildtype, grade 2–3 ¹⁶
H3.3	H3.3 G34R/V and/or H3 K27M mutations	H3.3 G34R/V: young patients (<50 years) with glioma, IDH-wildtype ¹⁵ H3 K27M: diffuse glioma involving the midline ^{15,16}

The *CDKN2A* and *CDKN2B* genes encode for proteins inhibiting the cell-cycle and regulates Rb1 and p53-dependent signaling, thus acting as tumor suppressors ¹⁵. Glioblastomas commonly have genetic alterations in *CDKN2A* and *CDKN2B*, often deletions ². These deletions are described to be associated with enhanced cell proliferation and a shorter median OS in IDH-mutant tumors ²², and tumors with *CDKN2A* and/or *CDKN2B* deletions are classified as WHO grade 4 ².

The most common numerical genetic alteration in glioblastoma, IDH-wildtype is a combined whole or partial chromosome 7 gain and chromosome 10 loss ^{2,23}. The chromosome 7 gain results in amplification of the epidermal growth factor receptor (*EGFR*) gene located on the short arm of chromosome 7. The *EGFR* signaling pathway is important in proliferation, differentiation, tumor growth and cell survival ^{3,24}. Overexpression of *EGFR* is found in a majority of glioblastoma, IDH-wildtype with amplifications in approximately 40% of the tumors ^{3,24,25}. In addition to amplifications, mutations and rearrangements of *EGFR* are also seen ^{24,25}.

Mutations in the telomerase reverse transcriptase (*TERT*) promoter are frequent in glioblastoma, IDH-wildtype and is a molecular marker for this diagnosis ¹⁵. *TERT* is involved in telomere stability and cellular proliferation ^{26,27}.

A diffuse glioma, IDH-wildtype, grade 2–3 with one or more of chromosome 7 gain and chromosome 10 loss, *EGFR* amplification and *TERT* promoter mutations meet the criteria for glioblastoma, IDH-wildtype and these analyses are therefore recommended to identify glioblastoma, IDH-wildtype ¹⁶.

Mutations in histone H3 are analyzed to identify certain subtypes of gliomas ¹⁵.

MGMT promoter methylation status

The DNA repair enzyme MGMT removes alkyl groups from the O⁶ position of the nucleotide guanine. If the levels of MGMT are reduced, the DNA lesions remain unrepaired leading to cytotoxicity and apoptosis. The *MGMT* gene can be epigenetically silenced through methylation of the promoter. Alkylating chemotherapy, for example temozolomide, damages DNA through adding alkyl groups. A methylated *MGMT* promoter is associated with reduced DNA repair and consequently a better response to alkylating chemotherapy. Analysis of

MGMT promoter methylation status is performed in clinical routine and influences treatment decisions especially for patients with glioblastoma, IDH-wildtype that are elderly or have worse WHO PS ^{15,28-31}.

The association between a methylated *MGMT* promoter and a better response to temozolomide is not always observed, and when studying both *MGMT* promoter methylation and *MGMT* protein expression the results are not always consistent ³². Several mechanisms are suggested to explain this, including methodological limitations in the analyses, different locations of the methylations in the promoter or in the gene body, upregulation of *MGMT* induced by temozolomide, and MMR deficiency. To achieve a more accurate prediction of the expected response to alkylating chemotherapy, analysis of both promoter methylation status and protein expression is suggested ³².

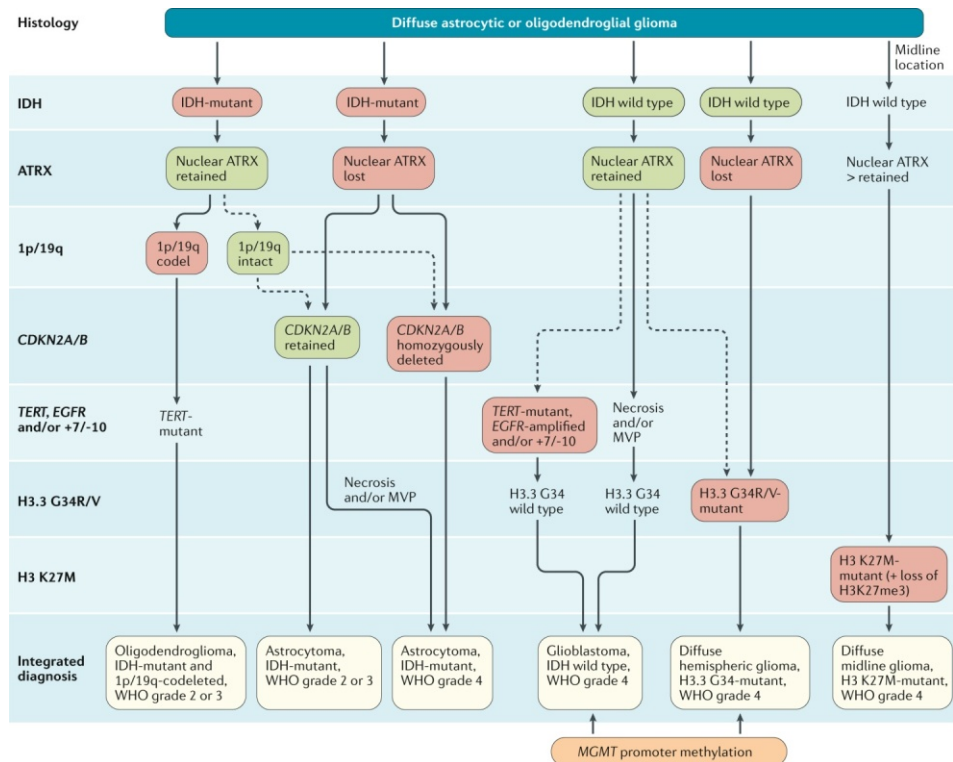


Figure 2 Schematic view of the classification of diffuse astrocytic or oligodendroglial tumors. Reprinted from Weller et al ¹⁵ (publication license CC BY 4.0, <http://creativecommons.org/licenses/by/4.0/>).

Glioblastoma, IDH-wildtype

At histopathologic examination, a glioblastoma, IDH-wildtype is composed of astrocytic poorly differentiated cells and is characterized by a high cellular pleomorphism with a variability in cell and nuclear size, shape and staining; nuclear atypia with abnormal appearance of cell nuclei; visible mitoses; microvascular proliferation with rapid blood vessel growth and necrosis that is often surrounded by a layer of palisading glioma cells; and an infiltrative growth pattern into healthy brain parenchyma ^{2,3}.

The diagnostic criteria for glioblastoma, IDH-wildtype in WHO CNS5 are; an IDH-wildtype, H3-wildtype, diffuse astrocytic glioma with one or more of the following; microvascular proliferation, necrosis, *TERT* promoter mutation, *EGFR* gene amplification, and +7/−10 chromosome copy number alterations ².

Treatment of glioma

The choice of treatment of a glioma depends on tumor type, WHO grade, the localization of the tumor and patient-related factors such as comorbidities, WHO PS and the patient's wishes. Treatment decisions should be discussed in a multidisciplinary tumor board with participation of neuroradiologist, neuropathologist, neurosurgeon, neurologist, neurooncologist and contact nurse.

The patient's PS is assessed according to the Eastern Cooperative Oncology Group (ECOG) PS scale from 0–5 (Table 3) ³³. The ECOG PS scale is often called WHO PS and, in this text and in the papers, WHO PS is used. For chemotherapy and radiotherapy, a WHO PS of 2 or better is most often required to cope with the treatment.

Table 3 Description of the ECOG Performance Status Scale ³³. Credit: The ECOG Performance Status Scale was developed by the Eastern Cooperative Oncology Group (ECOG), now the ECOG-ACRIN Cancer Research Group, and published in 1982. ecog-acrin.org/scale.

Grade	ECOG/WHO Performance Status Scale
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g. light housework, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair
5	Dead

Treatment of a patient with a glioma is often multimodal. In most cases, surgery is the first part of the treatment. The aim of glioma surgery is to safely remove as much as possible of the tumor to reduce symptoms and improve survival, and to secure tumor tissue for diagnostic analyses ¹⁶. Depending on the tumor location, a tumor resection may sometimes not be safe wherefore a biopsy is to prefer. If the tumor is located in an eloquent area of the brain, surgery can be associated with large risks for loss or impairment of neurological function. The eloquent areas are parts of the brain where an injury results in disabling neurological deficits ³⁴. Intraoperative mapping techniques might lead to safer control of the risks of neurological deficits ³⁵, and surgery can, in selected cases with a supratentorial tumor, be performed with the patient awake ³⁶. Minimally invasive surgery can be an optional treatment, especially for small lesions with a deeper location. One minimally invasive method is laser interstitial thermal therapy (LITT) when a catheter is introduced into the lesion, and the tissue is destroyed using heat ^{16,37,38}.

Surgery is the first part of the treatment for patients with glioma, WHO grade 2 and there is clear evidence for the benefit of early surgical resection for glioma, IDH-mutant, WHO grade 2 ³⁹. Further postoperative treatment is based on risk criteria. Patients who are

considered low-risk (e.g. young patients with a gross total resection or partial resection and without neurological symptoms) are followed by active surveillance and patients with high-risk criteria are recommended direct postoperative radiotherapy and/or chemotherapy with procarbazine, lomustine and vincristine (PCV) or temozolomide.

For patients diagnosed with an astrocytoma, IDH-mutant, WHO grade 3 surgery is followed by radiotherapy and adjuvant temozolomide ⁴⁰ and for oligodendroglioma, IDH-mutant and 1p19q-codeleted, WHO grade 3, surgery is often followed by radiotherapy and chemotherapy with PCV ^{15,16}.

The initial treatment of patients with glioblastoma and astrocytoma, IDH-mutant, grade 4 is surgery with maximal safe tumor resection. To enhance the extent of tumor resection, the prodrug 5-aminolevulinic acid (5-ALA) can be administered preoperatively. After administration, 5-ALA is metabolized to fluorescent porphyrins that accumulate in glioma cells. During surgery, the fluorescence is visualized using a microscope with ultraviolet light which guides tumor resection. The use of 5-ALA is demonstrated to enhance the extent of tumor resection and to prolong progression free survival (PFS) and survival in glioma, WHO grade 3–4 ⁴¹⁻⁴³.

For patients below approximately 65–70 years, and in good WHO PS, surgery is followed by radiochemotherapy. The radiotherapy is delivered externally with photons in 2 Gray (Gy) fractions to a total dose of 60 Gy to the resection area with a margin. Chemotherapy with the oral alkylating agent temozolomide is administered daily during radiotherapy and, after completion of radiotherapy, in monthly 5-day courses during six months ^{44,45}. For patients with worse WHO PS and/or elderly patients, treatment is determined based on *MGMT* promoter methylation status where a shorter course of radiochemotherapy (40.05 Gy in 2.67 Gy fractions) ^{29,46} or temozolomide as monotherapy ²⁹ are recommended treatment options for *MGMT* promoter methylated tumors. For tumors without *MGMT* promoter methylation, two weeks of radiotherapy (34 Gy in 3.4 Gy fractions) without chemotherapy is recommended ¹⁶. Hypofractionated radiotherapy (25 Gy in 5 Gy fractions) or best supportive care may be the best treatment option in selected cases ¹⁶. For patients in good WHO PS, inclusion in available clinical studies is recommended.

The latest addition to standard treatment for glioma, WHO grade 4 is tumor treating fields (TTFields) that was introduced in Sweden in 2019.

Through arrays placed on the skull, low intensity alternating electrical fields are delivered to the tumor. TTFields impairs mitosis, but several additional mechanisms of action are suggested ⁴⁷. Clinical trials with TTFields have reported both longer OS and PFS ⁴⁸⁻⁵⁰. TTFields is recommended for patients operated for a supratentorial glioma, WHO grade 4 and without clinical signs of tumor progression after radiochemotherapy, for use until second progression of the tumor, or a maximum of two years ¹⁶. TTFields is also studied in clinical routine after approval, where the percentage of time that TTFields is used is reported to be important for the effect and that a decline in usage was seen after the first months of treatment ^{48,51}. Since TTFields was introduced in Sweden in 2019 (four years after the study period), no patients in our study have been treated with TTFields.

Despite the extensive initial treatment, there is in most cases a progression or recurrence of the tumor. After initial treatment, the patient is monitored with clinical investigations and MRI, every 2–3 months. At tumor progression there are unfortunately few evidence-based treatment options. The patient should be discussed in a multidisciplinary tumor board and assessed for new surgery, radiotherapy and/or chemotherapy ¹⁶. Treatments used at tumor progression are temozolomide, nitrosourea (e.g. lomustine), and the vascular endothelial growth factor (VEGF) A-inhibitor bevacizumab ^{15,52}. Clinical trials with bevacizumab have shown effect on PFS, but not on OS ⁵³. At tumor progression, no survival benefit was shown from the checkpoint inhibitor nivolumab compared to bevacizumab ⁵⁴, or TTFields compared to physician's choice of treatment ⁵⁵. Treatment after tumor progression is evaluated approximately every 2–3 months clinically and with neuroradiology.

All patients should be offered a contact nurse early during the treatment. The contact nurse acts as a coordinator for the patient and the patient's relatives. Cognitive deficits are common among brain tumor patients and potentially affect the patients' abilities to communicate with the caregiver and to be involved in the treatment, which makes the contact nurse even more important.

The individual patient's need for cancer rehabilitation should be assessed during and after treatment of a cancer, including brain tumors. The aim of cancer rehabilitation is to maintain or restore functions that are impaired by the tumor or the treatment. During cancer rehabilitation different professionals are involved, for example physiotherapist,

occupational therapist, health care counselor/ health care social worker, and sexologist depending on need ⁵⁶.

Special considerations during glioma treatment

During the initial part of the postoperative treatment, the radiographic phenomenon pseudoprogression may occur. A pseudoprogression is a contrast enhancement within the radiation field that appears during or after radiotherapy and disappears with time without change of treatment ⁵⁷. Pseudoprogression is seen in 30–40% of glioma, WHO grade 4 and is more prevalent in gliomas with *MGMT* promoter methylation ^{58,59}. When a pseudoprogression is suspected, the recommendation is often to continue initial treatment until the next radiological evaluation ⁵⁹.

In addition to MRI, amino-acid PET may be used in selected cases, for example to differentiate true progression from pseudoprogression. In other malignancies, the tracer fluorodeoxyglucose (FDG) is widely used for diagnostic purposes and treatment evaluation. It is difficult to discriminate a brain tumor from normal brain tissue using FDG-PET since the healthy brain has a high glucose uptake ⁶⁰. Therefore, other tracers with lower uptake in normal brain tissue, such as amino acids are used. Clinical studies of different amino acid tracers are ongoing (e.g. FLT (¹⁸F- fluorothymidine) and FET (O-(2-[¹⁸F]fluoroethyl)-L-tyrosine)).

The BBB affects the passage of molecules, for example many drugs, to the CNS. The BBB is a protecting layer of microvascular endothelial cells connected by adherence junctions that separates the blood from the interstitial fluid in the CNS and regulates the entrance and expulsion of molecules. Efforts have been made to regulate BBB permeability and to modify drugs to enhance the passage over the BBB. Radiographic contrast is normally not penetrating the BBB, and the contrast enhancement seen in high-grade glioma is associated with a disrupted BBB which could also facilitate drug entrance. In glioma, the tumor has both contrast-enhancing and non-enhancing parts and BBB permeability is thus important for brain tumor treatment and is one important reason for the difficulties in finding effective treatments ^{61,62}.

Prognostic and predictive factors

A prognostic factor provides information on expected survival (or another health outcome) for a patient with a specific disease irrespective of treatment. A predictive factor gives information about the patients' expected response to a certain treatment or intervention.

Many of these prognostic and predictive factors are biomarkers. A biomarker is, by the United States Food and Drug Administration (FDA), defined as a characteristic that is measured as an indicator of a normal biological process, a pathogenic process, or the response to an exposure or intervention, including therapeutic interventions. In addition to providing prognostic or predictive information, biomarkers can indicate a risk for a disease or condition, offer diagnostic information or can be used for monitoring a treatment. There are several types of biomarkers: molecular, histologic, radiographic, or physiologic characteristics ⁶³.

Prognostic or predictive factors in glioma could be patient-related, tumor-related, or treatment-related. Clinical factors are both the patient-related, and treatment-related. In the following section, clinical and tumor-related prognostic and predictive factors, sociodemographic factors and metabolic markers in glioma, WHO grade 4 are described.

Clinical and tumor-related prognostic factors

Clinical factors associated with a longer OS in glioma, WHO grade 4 are low age at diagnosis, good WHO PS, a more extensive tumor resection, and more intensive postoperative treatment ^{10,13,64-68}.

Molecular pathological factors associated with prognosis are IDH mutations, *MGMT* promoter methylation status, *TERT* promoter mutations and *CDKN2A* deletions. IDH mutations does not affect the prognosis within each diagnosis since the diagnoses are defined by IDH status. However, there are prognostic differences between the two diagnoses with a better prognosis for astrocytoma, IDH-mutant, grade 4 ^{10,69}. Glioma, WHO grade 4 with a methylated *MGMT* promoter have a favorable prognosis ³¹. Glioma, WHO grade 3–4 with mutations in the *TERT* promoter have a shorter OS and a shorter PFS ^{26,27}, and a homozygous deletion of *CDKN2A* is related to a shorter OS in astrocytoma, IDH-mutant ^{22,70} and indicates a WHO grade 4 tumor ².

Clinical and tumor-related predictive factors

A methylated *MGMT* promoter is a positive predictive marker for treatment with alkylating agents, for example temozolomide ^{28,30} and lomustine, and radiotherapy ⁷¹. A mutation in the *BRAF* gene encoding the proto-oncogene B-raf, the *BRAF* V600E mutation, is associated with response to treatment with BRAF and MEK inhibitors ⁷²⁻⁷⁴.

The potential role of IDH status as a predictive factor in glioma, WHO grade 4 is not yet known. Using IDH mutation as a predictive factor,

treatment with the IDH inhibitor vorasidenib improved PFS and time to next intervention (TTNI) for patients with recurrent or residual glioma, IDH-mutant, WHO grade 2 ⁷⁵. Data from small series of glioma, WHO grade 3–4 patients are reported ⁷⁶ and prospective studies are ongoing.

Sociodemographic factors

Sociodemographic factors are characteristics such as age, sex, educational level, employment status, income and cohabitation status that are used to describe individuals or a group. Sociodemographic factors are known to influence health within and between countries. In Sweden, the National board of Health and Welfare report differences in incidence, tumor stage at diagnosis and survival for several types of cancers in areas with sociodemographic differences based on educational level, economic standard, rates of unemployment and the need for allowances ^{77,78}.

In Sweden, the health care system is largely public, and health care should, by law, be offered equally to all patients regardless of sociodemographic differences. The health care is to a large extent paid by regional and municipal taxes and patient fees cover only a small part of the costs.

Socioeconomic status

Socioeconomic status (SES) is a way of describing a person's position in the society and can be estimated in different ways. Common proxies for SES, that can be used alone or in combination, are education, occupation, income, and area of living. Different estimates of SES does not always correlate with each other, for example a high educational level is not always associated with a high income, and some measures of SES might vary over time for an individual ⁷⁹. The highest completed educational level is commonly used as a proxy for SES and is used in Study II.

The impact of SES on diagnosis, treatment and prognosis is studied for many cancers. Participation in cancer screening programs differs with a lower rate of participation for persons with lower SES both in Sweden and internationally ^{80,81}. Glioma incidence was previously studied in relation to SES but with diverging results where some studies report higher incidence of glioma with higher SES ^{82,83} but other studies have not found any association ^{84,85}. SES is also described to be related to treatment in glioma with a lower probability of a more extensive

treatment for patients with older age and lower SES ⁸⁶, and shorter waiting times for surgery for glioma, WHO grade 2 patients with higher income or education ⁸⁷. Previous studies have reported a better survival for brain tumor patients with higher SES ^{84,88-91}.

Cohabitation status

A cohabitant person is living together with someone, and the cohabitants often provide support to each other. To assess the impact of the support from a cohabitant on stage at diagnosis, treatment and prognosis, marital status is studied for different cancers. These studies have shown an association between marriage and earlier stage at diagnosis, a larger proportion of treatment with curative intent and better disease-specific survival for several cancers not including brain tumors ⁹². Since many persons are living together without being married, studying cohabitation status instead of marriage could better reflect the social support that the patient can get from their cohabitant. Glioma patients may also potentially benefit more than other patient groups from being a cohabitant since the disease can affect cognitive functions which may result in difficulties in communicating with health care and in participating in treatment decisions. For glioma, WHO grade 4, a survival advantage has been reported for married patients ⁹³ but in another study including CNS tumors, no survival differences were found between married and divorced patients ⁸⁴.

Travel time to health care

The travel time required to receive health care might affect cancer care and treatment. The implications on treatment of travel time are studied for several cancers, but the studies regarding glioma are sparse. The number of visits at the medical center was found to be lower for breast cancer patients with a longer travel distance ⁹⁴. Treatment choices can be affected by the patient's travel time, for example to adjust the treatment to avoid radiotherapy in prostate cancer ^{95,96} or breast cancer ⁹⁷. Long travel time is also described as a reason for patients to not participate in clinical trials ⁹⁸. One previous study of rural and urban residents found small survival differences for glioma, WHO grade 4 and oligoastrocytoma with a worse prognosis in the rural group ⁸⁸. In another study, no differences were found regarding the access to neurosurgical and oncological treatment or waiting times in relation to travel distance ⁹⁹.

Region of residence

In Sweden, there are differences between the Northern and Southern regions with aspect to population density and travel times to health care. The northern Sweden health care region covers approximately half of the area of Sweden and is more sparsely populated with around 10% of the population. There are regional differences in educational attainment in Sweden, with a lower educational level in rural areas ¹⁰⁰. Regional differences in incidence and survival for many cancers in Sweden have been described, with brain tumors studied together as one entity ⁴.

These geographic, demographic and socioeconomic differences make a regional comparison between northern and southern Sweden important to study potential differences in prognosis for glioma patients. The northern Sweden healthcare region consists of the four northernmost regions in Sweden: Norrbotten, Västerbotten, Jämtland-Härjedalen and Västernorrland.

Metabolic markers

Metabolic alterations are one of the hallmarks of cancer ^{101,102}. The metabolism is all processes that provide energy and the basic building blocks of cells and metabolize exogenous substances. Involved in all metabolic processes are the metabolites: small molecules such as sugars, amino acids and nucleotides that are found in a biological sample. Together all metabolites constitute the metabolome ¹⁰³ that is constantly growing with the number of substances that an organism is exposed to. Today approximately 220 000 metabolites are included in the Human Metabolome Database (HMDB) ¹⁰⁴.

Metabolomics

Omics-technologies are widely used for comprehensive analyses in research and include genomics, transcriptomics, proteomics and metabolomics. Metabolomics aims at studying physiological and pathological processes in cells, bio-fluids and tissues through large-scale analysis of metabolites ¹⁰⁵. The metabolite concentrations measured in the analysis are affected by the activity of the enzyme producing them, the supply of substrates, the concentrations of cofactors or products and other regulatory processes ¹⁰³. These processes and the factors regulating them make the metabolome dynamic. Compared to the genome, transcriptome and proteome, the metabolome is located further

downstream of the signal-transduction pathway and therefore reflects the phenotype and function to a larger extent ¹⁰⁵.

There are different methods for metabolite analyses. In the targeted approach, the analysis is set up to detect a smaller number of known metabolites resulting in a more accurate identification and quantification of these metabolites. In contrast, the untargeted method is a broad analysis aiming at a comprehensive characterization and relative quantification of metabolites. The first step in the metabolite analysis is the handling and preparation of the biological sample to block the metabolic processes in the tissue and maintain the concentrations of the metabolites. The next steps are, for some methods including gas-chromatography (GC) and liquid-chromatography (LC) based mass spectrometry (MS), to separate, ionize and then analyze the metabolites. There are different methods for separation based on the mass to charge (m/z) ratio, and ionization of metabolites. The methods for separation, ionization and analysis are possible to combine in different ways that are suitable for the planned analysis. A challenge in the metabolite analysis is to obtain both the highest possible resolution and sensitivity ¹⁰⁵.

Metabolite studies in glioma

Previous metabolite studies in glioma, WHO grade 4 have reported metabolic changes in blood samples several months to years before diagnosis ¹⁰⁶⁻¹⁰⁸, metabolic differences between glioma subtypes ^{109,110}, and in relation to IDH status ¹⁰⁹ and *MGMT* promoter methylation status ¹¹¹. Metabolic changes are described during radiotherapy and treatment with chemotherapy and/or bevacizumab ¹¹²⁻¹¹⁴, as well as at tumor progression ¹⁰⁶. Metabolic differences related to prognosis are also reported ^{110,115-117}. Metabolite analyses in glioma are performed both in tumor tissue, in micro dialysate from the tumor and from brain adjacent to tumor, and in blood samples. Changes in different metabolic pathways are described including carbohydrate, lipid, amino acid and nucleotide metabolism, and growth factor signaling pathways ^{105,118}.

Aims

The overall aim of this work was to enhance the knowledge on prognostic and predictive factors in glioma with a special emphasis on glioblastoma IDH-wildtype and astrocytoma, IDH-mutant, grade 4. The thesis investigates prognostic and predictive clinical and sociodemographic factors, and metabolic markers.

Specific aims

- I. To evaluate if the survival has improved over time for patients with glioma, WHO grade 4 at a single regional center.
- II. To analyze the possible association between glioma patients' survival and sociodemographic factors.
- III. To find prognostic and predictive metabolic markers for glioblastoma IDH-wildtype and astrocytoma, IDH-mutant, grade 4.

Materials and methods

An overview of the materials and methods in the three studies that this thesis is based on is presented in Table 4.

Table 4 Overview of main research question, study design and outcome measures for the studies included in this thesis.

Study	I	II	III
Main research question	Has the survival for glioma, WHO grade 4 patients improved over time in northern Sweden?	Does socioeconomic status, travel time to health care, cohabitation status or region of residence impact survival for glioma patients in Sweden?	Can metabolic markers give information regarding prognosis and treatment response in glioma, WHO grade 4?
Type of study	Retrospective cohort study	Register based retrospective cohort study	Retrospective cohort study
Data source	Clinical database, the Swedish Cancer Registry	RISK North database	Clinical database, brain tumor biobank
N	571 for survival analysis 244 for treatment analysis	1276	141 glioblastoma, IDH-wildtype 9 astrocytoma, IDH-mutant, grade 4
Inclusion criteria	Diagnosis of glioma, WHO grade 4 and residing in the northern Sweden health care region	Diagnosis of glioma, WHO grade 1–4 with data in the National Quality Registry for CNS Tumors and residing in a region with high registry coverage	Diagnosis of glioblastoma, IDH-wildtype or astrocytoma, IDH-mutant, grade 4, with a tumor tissue sample from primary surgery in the biobank
WHO classification	1 st edition 1979 2 nd edition 1993 3 rd edition 2000 4 th edition 2007	4 th edition 2007	Revised 4 th edition 2016
Study years	1995–2015 for survival analysis 2005–2015 for treatment analysis	2009–2013	2005–2015
Main outcome measures	Differences in median overall survival	Differences in median overall survival	Differences in median overall survival and time to next intervention

Patients (I–III)

Studies I and III are based on a biobank that was initiated in Umeå in 2005, collecting blood samples and tumor tissue samples for patients undergoing surgery for a brain tumor at Umeå University Hospital. In 2010 the Uppsala/Umeå Comprehensive Cancer Consortium (U-CAN) project was initiated ¹¹⁹, and the biobank is since then part of U-CAN. U-CAN is a collaboration initiated by Uppsala and Umeå Universities involving several universities and hospitals in Sweden with the aim to collect biomaterials and data for cancer research.

For Studies I and III, adult patients in the northern Sweden health care region with a histopathological diagnosis of an intracranial glioma, WHO grade 4 were identified through the Swedish Cancer Registry, the medical records from the departments of Neurosurgery and Oncology at Umeå University Hospital and the database of the biobank.

In Study I, all 571 patients in the northern Sweden health care region with a diagnosis of glioma, WHO grade 4 during 1995–2015 were included to study survival. The patients were split into four groups according to year of diagnosis, with the cutoff points chosen considering the following changes in treatment or classification:

- 1997: the implementation of the 2nd edition of the WHO classification
- 2005: the introduction of radiochemotherapy in clinical routine
- July 2010: the use of 5-ALA at surgery

A cohort of 244 patients with a tumor tissue sample in the biobank was studied more in detail in Study I. This group is named the biobank cohort. The study cohort in Study III consisted mainly of patients from the biobank cohort. See Figure 3 for an overview of the different patient cohorts in Studies I and III.

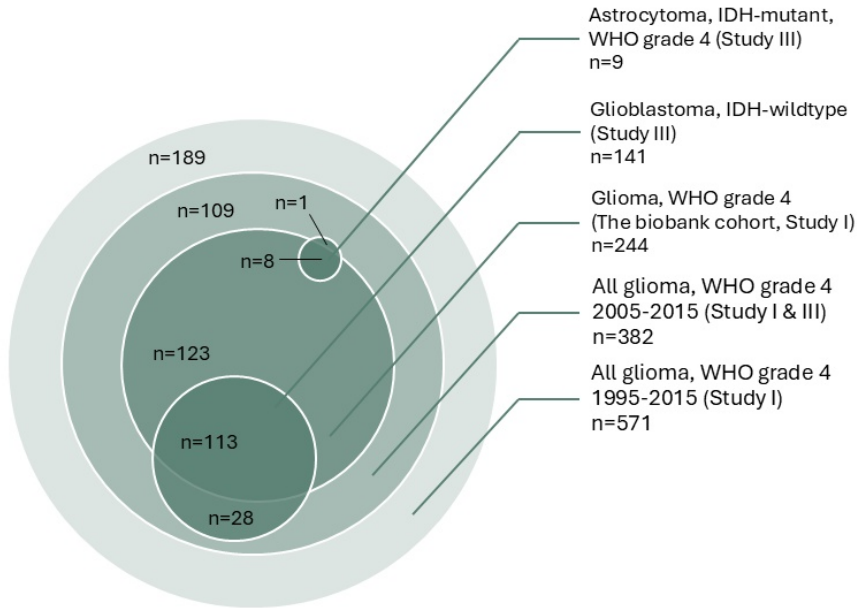


Figure 3 Venn diagram illustrating the different patient cohorts in Studies I and III.

In Study II, the study cohort consisted of all 1276 patients with a diagnosis of glioma, WHO grade 1–4 registered in the National Quality Registry for CNS Tumors between 2009 and 2013 and living in regions with a high coverage in the quality registry (Middle Sweden, Stockholm-Gotland, Southeastern and the North). The patients were selected by the International Statistical Classification of Diseases and Health Related Problems (ICD)-7 code 193 (malignant neoplasm of the brain and other parts of the nervous system), and to identify glioma, WHO grade 4 patients the Systematized Nomenclature of Medicine (SNOMED) code 94403 was used.

Collection of clinical data (I, III)

For Study I, data regarding age, sex and survival for all 571 patients with glioma, WHO grade 4 were retrieved from the Swedish Cancer Registry.

For the biobank cohort (Study I) and the study cohort in Study III, clinical data were collected from medical records (NCS Cross[®] (EVRY, Stockholm, Sweden), VAS[®] (Norrbottens läns landsting, Luleå, Sweden), and Cosmiq[®] (Cambio Healthcare Systems, Stockholm, Sweden).

Information on age at diagnosis, sex, WHO PS, comorbidities, postoperative radiotherapy and/or chemotherapy, and treatment at tumor recurrence/progression were collected.

The tumor location was registered and defined as frontal, parietal, temporal, occipital, central, multifocal, or located in the posterior fossa. Location in eloquent areas was also registered, and the following areas were considered eloquent: motor or sensory cortex, visual cortex, speech areas, internal capsule, basal ganglia, hypothalamus or thalamus, brain stem, and dentate nucleus.

Postoperative treatment was categorized in five subgroups:

1. Completed radiochemotherapy: radiotherapy to 60 Gy in 2 Gy fractions with concomitant daily temozolomide (75 mg/m²) followed by six or more monthly five days cycles of adjuvant temozolomide (150–200 mg/m²). Dose-reduction of temozolomide was allowed.
2. Interrupted radiochemotherapy: patients scheduled for radiochemotherapy but interrupting the treatment. The reason for interrupting the treatment was registered and categorized as tumor progression, treatment-related toxicity or a combination of both.
3. Other treatment: temozolomide or radiotherapy as monotherapy, hypofractionated radiotherapy with adjuvant temozolomide, or treatment in clinical trials including radiotherapy and/or temozolomide.
4. Clinical trials with bevacizumab ¹²⁰ or valganciclovir ^{121,122} in combination with radiotherapy and temozolomide.
5. Best supportive care: patients that did not receive radiotherapy nor chemotherapy.

All patients scheduled for radiochemotherapy constitute the intention to treat (ITT) radiochemotherapy group.

In Study III, metabolite data were investigated in relation to survival and treatment response. To estimate treatment response, TTNI after primary treatment was registered. The primary treatment was defined as surgery and postoperative radiotherapy and/or chemotherapy. TTNI was registered as the timepoint when a change of treatment or the decision to interrupt treatment was made due to clinical and/or radiological signs of tumor progression. When a pseudoprogression was suspected, TTNI was registered as the later timepoint when a clinical decision to change or

interrupt treatment was made. Death that was judged to be caused by tumor progression was registered as TTNI but death of other causes (e.g. infection, thrombosis, complications after surgery) was censored.

Treatment at tumor recurrence/progression after primary treatment was registered and categorized as surgery, systemic therapy (chemotherapy and/or bevacizumab), radiotherapy or best supportive care.

Survival was, for all patients, calculated from the date of diagnosis, defined as the date of the pathology report, to the date of death. Patients that were alive at the last timepoint of data collection were censored, for Study I 3 September 2017, for Study II 31 December 2014, and for Study III, 17 February 2021. In all studies, median OS was used in the survival analyses.

Volumetric measurements of tumor volume (I, III)

The contrast-enhancing tumor volumes were measured on preoperative and postoperative MRI- or CT-scans to calculate the extent of tumor resection. The SECTRA[®] (Sectra, Linköping, Sweden)-system was used for measuring contrast-enhancing lesions on T1-weighted MRI-sequences with gadolinium contrast. Axial images were used for delineating the lesions, the area from each slice was multiplied with slice thickness to calculate the volume, and the volumes of all slices were summarized to obtain total tumor volume. If no MRI was available, or the MRI was performed more than three weeks before surgery, a CT-scan was used for the measurements of tumor volume.

The change in contrast enhancing tumor volume between the preoperative and postoperative examination was calculated. Surgery was categorized according to the reduction of contrast enhancing tumor volume into tumor resection $\geq 95\%$, tumor resection $< 95\%$ or biopsy. When a biopsy was performed, it was reported by the surgeon and the reduction in tumor volume was estimated to 0%.

Collection of clinical and sociodemographic data (II)

The RISK North database (II)

The RISK North database was set up at the Regional Cancer Centre in Umeå to study sociodemographic factors in relation to cancer treatment and survival. The RISK North links the National Quality Registries for

CNS tumors, colorectal cancer, and esophageal and gastric cancer with databases with demographic and health care data ¹²³. All patients registered in the National Quality Registry for CNS Tumors between 2009–2013 are included in RISK North.

For Study II, data from the National Quality Registry for CNS Tumors, the longitudinal integration database for health insurance (LISA), the total population registry (TPR), and the geography database (GD) were used. The LISA, the TPR and the GD are administered by the governmental agency Statistics Sweden.

The National Quality Registry for CNS Tumors (II)

The Swedish National Quality Registries were created to facilitate the evaluation and follow-up of health care outcomes and quality. Today there are 30 national quality registries for different cancers. The National Quality Registry for CNS Tumors have the intention to register all primary brain tumors in adults. Since 2018 tumors in the spinal cord and meninges are included and both tumors diagnosed from histopathology and radiology should be registered, but not tumors diagnosed at autopsy. Before 2018 the registry was called the National Quality Registry for Brain Tumors. Registry coverage is evaluated through comparison with the National Cancer Registry where it is mandatory to register all detected cases of cancer. The National Quality Registry for CNS Tumors registers information regarding diagnosis, surgery, postoperative radiotherapy and chemotherapy including information on lead times and patient reported outcome measures (PROM) ¹²⁴.

For Study II, data regarding age at diagnosis, sex, smoking status, WHO PS, and type of surgery were retrieved from the quality registry. Surgery was categorized as biopsy, partial resection or gross total resection. The extent of tumor resection (partial or gross total) was estimated from a postoperative MRI.

All regions with a high coverage in the quality registry during the studied years 2009–2013 were included in Study II (Middle Sweden, Stockholm-Gotland, Southeastern and the North, all with a coverage >97%), but the regions with a lower coverage (South 4–79%, and West 88–98%) were excluded from the analyses. In the regional analyses, the North was compared to the southern regions (Middle Sweden, Stockholm-Gotland and Southeastern).

The longitudinal integration database for health insurance (II)

The LISA registers information on education, civil status, occupation, disposable income and sick leave for persons aged ≥ 15 years in Sweden (before 2010 persons aged ≥ 16 years) ¹²⁵. The LISA was initiated in 1990 to facilitate research and evaluation regarding health and disease related to health insurance and the labor market. For Study II, data on educational level were retrieved from the LISA. Educational level was divided into three categories: middle school (up to nine years of compulsory school), high school (secondary education of two or three years), and university/college.

The total population registry (II)

The TPR registers data on birth, death, marital status, and migration for all inhabitants in Sweden. For Study II, data on cohabitation status were obtained from the TPR. Being a cohabitant was defined as living together with another adult person.

The geography database (II)

The GD registers the geographical coordinates of the home address for all persons residing in Sweden. For Study II, the GPS coordinates for the patient's home address were retrieved to calculate the travel time by car to the closest regional hospital.

Molecular pathological analyses (I, III)

IDH mutation analysis (III)

Analyses of IDH mutations were not part of the routine diagnostic procedure during the early study period. However, for Study III, IDH mutation analysis was performed for reclassification of the tumors according to the revised 4th edition of the WHO classification. In the IDH analysis, the following mutations in *IDH1* and *IDH2* were detected: IDH1-R132H, IDH1-R132C, IDH2-R172K, and IDH2-R172M. A multiplex ligation-dependent probe amplification assay Po88-C2 (lot C2-0416) was performed according to the manufacturer's instructions (MRC-Holland, Amsterdam, The Netherlands). The results were interpreted by an expert geneticist.

MGMT promoter methylation analysis (I, III)

MGMT promoter methylation analysis was performed for a subgroup of the patients in the ITT radiochemotherapy group in Study I and for all patients in Study III. The cutoff for clinically relevant *MGMT* promoter methylation is dependent on the method of analysis^{16,126}. In our analysis, *MGMT* promoter methylation status was graded as <10%, 10–25% or >25% methylation. In Studies I and III, *MGMT* promoter methylation $\geq 10\%$ was defined as methylated and methylation <10% was defined as unmethylated according to the definition used in clinical routine.

The analysis was performed through bisulfite conversion of 50 ng genomic DNA using the EpiTect bisulfite kit (Qiagen, Hilden, Germany), including a 50% methylated sample and a negative control. The theascreen *MGMT* Pyro Kit together with Pyromark Q24 (Qiagen, Hilden, Germany) was used for pyrosequencing. Bisulfite conversion and pyrosequencing were performed according to the manufacturer's instructions.

Handling of tumor tissue samples and metabolite analysis (III)

The tumor tissue samples in the biobank were collected at surgery after securing tumor tissue for the diagnostic analyses. The tumor tissue samples were stored at -80°C within 30–60 minutes from sampling.

Metabolite extraction and mass spectrometric analysis

Frozen tissue was used for extraction of metabolites. The tumor tissue was homogenized, and proteins were precipitated. Extracted metabolites were then speed-vacced to remove solvents and stored at -80°C .

Untargeted GC-MS and LC-MS analyses were performed. To minimize confounding, the run order was randomized with respect to storage time in freezer and patient factors. Quality control samples were included in the analysis. Missing values below the detection limit were assigned to half-minimum values and metabolites were excluded if >10% of the data was missing. The analysis yielded the concentrations of 1132 metabolic features, out of those 240 were identified with high confidence.

Statistical analyses (I–III)

For Studies I and III, a database with clinical data was created using MS Excel (Microsoft Corp, Redmond, WA). Statistical analyses were performed using SPSS 24 and SPSS 27 (IBM Corp, Armonk, NY) (Papers I and III), and R version 3.6.0 (R Core Team, Vienna, Austria) (Paper II). All tests were two-tailed, and results were considered significant when $p < 0.05$.

Study I

Chi-square test was used to test for differences in proportions, Student's t-test to compare differences between means, and Mann Whitney U-test to test for differences between medians between groups. Univariable survival analysis was performed using Kaplan-Meier estimates and the subgroups were compared with log-rank test. Multivariable survival analysis was performed using Cox proportional hazard analysis to estimate hazard ratios (HR) with 95% CI to analyze the association between survival and age, preoperative WHO PS, comorbidities, extent of surgery and postoperative treatment in the biobank cohort. The results were displayed in a forest plot.

Study II

Chi-square test was used to test for differences in proportions, and Student's t-test or analysis of variance (ANOVA) to assess differences between means between groups. Multivariable survival analysis was performed using Cox proportional hazard analysis to estimate HRs with 95% CI to analyze the association between survival and sex, age at diagnosis, extent of surgery, education level, travel time to the regional hospital, cohabitation status, and region of residence. The results were displayed in forest plots.

Study III

Chi-square test was used to test for differences in proportions, and Mann Whitney U-test to test for differences between medians.

The metabolite concentrations were checked for normality through assessing skewness and kurtosis. A \log_2 -transformation of the metabolite concentration was performed if skewness or kurtosis was < -2 or > 2 . Univariable survival analysis was performed for the individual metabolite concentrations using Cox proportional hazard analysis to estimate HRs with 95% CI. $\log_2(\text{HR})$ and $-\log_{10}(\text{p-value})$ were displayed in volcano plots. For significance, a p-value < 0.05 and a HR < 0.91 or

>1.10 were required. Metabolites with HRs between 0.999–1.000 in the Cox proportional hazard analysis were excluded from the tables and volcano plots due to lack of effect size.

Metabolite scores were calculated based on the metabolites significantly associated with OS and TTNI in the Cox proportional hazard analysis. The prognostic score was based on all glioblastoma patients and included ten significant metabolites from the OS analysis. For the predictive score, glioblastoma patients with resective surgery in the ITT radiochemotherapy group were included and the score was based on three significant metabolites from the TTNI analysis. The metabolite concentrations were split in quartiles, and the quartiles were assigned scores from 1 (shortest OS/TTNI) to 4 (longest OS/TTNI). The scores for the individual metabolites were summarized into a total score for each patient, and the patients were split into groups based on the total metabolite score. For OS the total score ranged between 10 and 40, and for TTNI between 3 and 12.

Univariable survival analyses were performed using Kaplan-Meier estimates with log-rank test. Multivariable survival analyses were performed using Cox proportional hazard analysis to estimate HRs with 95% CI for the prognostic and predictive scores to assess any association between survival or TTNI and metabolite score, sex, age at diagnosis, *MGMT* promoter methylation status, WHO PS and, for the prognostic score, extent of surgery. The results were displayed in forest plots.

Ethics

Ethical approval

The clinical studies were conducted in accordance with the World Medical Associations Declaration of Helsinki and were approved by the regional ethical board in Umeå.

For Studies I and III, ethical approval was obtained for the generation of the biobank, and the analyses of the tumor tissue samples (2003-03-154, 2006-06-124, 2010-219-31M, 2011-308-31M, 2016-95-32M, and 2016-188-32M). For Study II, the ethical board approved studying risk factors through linking of registries (2014/278-31).

Ethical considerations

For Studies I and III, most of the patients gave their written informed consent to participate in the study. For the patients where no written informed consent was available, the regional ethical board approved their inclusion in the study since the contribution from their data was considered important and the potential harm from the research was estimated to be very low. Obtaining an informed consent from patients with brain tumors might need special consideration since the tumors can impair cognitive function and decision making. Research involving patients with brain tumors, as well as other conditions with a risk for cognitive impairment, is important and necessary to enhance our knowledge of the diseases and to improve the treatment.

Individual information regarding a person's health and disease are sensitive personal data and must be handled with care. In the health care this data is covered by secrecy. During the period of this project, the interpretation of the laws that regulates access to clinical data has changed implying a stricter regulation during the later years. For research purposes, an application must be made for disclosure of sensitive personal data according to the patient data act and the general data protection regulation services. The clinical data that is the basis of Studies I and III were collected before the implementation of the new regulations. To protect anonymity, the study subjects were given individual study numbers and the link between the study number and the personal identity number is kept at the neurosurgical unit at Umeå University Hospital.

For Study II, the patients were not asked for informed consent to be registered in the quality register but must actively choose to not participate. In the sociodemographic registries all Swedish inhabitants are registered. A serial number was assigned to each individual, and this serial number was used for linking the individuals' data from the different registries. The analyses were performed on an pseudonymized dataset without possibility to link data to the actual individuals. This pseudonymized data set is kept at the Regional Cancer Centre North in Umeå.

All studies were observational meaning that no intervention was part of any of the studies and the patients were treated according to the standard of care or within a clinical trial.

Results

Results Study I

Patient characteristics

The study cohort included adult glioma, WHO grade 4 patients in the northern Sweden health care region. For survival analysis, all 571 patients diagnosed with a glioma, WHO grade 4 between the years 1995–2015 were studied. For the more detailed analysis in the biobank cohort, 244 patients with a diagnosis of glioma, WHO grade 4 between 2005–2015 were included. In total 382 patients had a diagnosis of glioma, WHO grade 4 during 2005–2015 and the biobank cohort thus represents 64% of all glioma, WHO grade 4 patients in the region during these years.

For all 571 patients and in the biobank cohort, the median age at diagnosis was 64.4 years (range 19.0–84.2 years for all patients, and 21.3–84.1 in the biobank cohort) and the gender distribution was similar in both cohorts (62% males among all patients and 64% in the biobank cohort). Most of the patients in the biobank cohort had WHO PS 0–1 (169 patients, 69%) (Table 5).

When comparing the biobank cohort (244 patients) with the 138 patients diagnosed with a glioma, WHO grade 4 during the same period but not part of the biobank cohort, the patients in the biobank cohort were slightly younger (median age at diagnosis 64.4 years (range 21.3–84.1) vs 67.3 years (range 41.5–84.2), $p < 0.01$) but the sex distribution did not differ between the groups.

Survival in the main study cohort

The median OS for all 571 patients was 9.3 months (95% CI 8.5–10.1), the 2-year survival was 13% and the 5-year survival was 3% (Table 5). To study survival over time, the cohort was divided into four subgroups based on year of diagnosis. The cut-off points were chosen according to the following changes in diagnostic and/or treatment routines: the implementation of a new WHO classification in 1997, the introduction of postoperative radiochemotherapy as standard treatment in 2005, and the use of 5-ALA during surgery in clinical routine in July 2010.

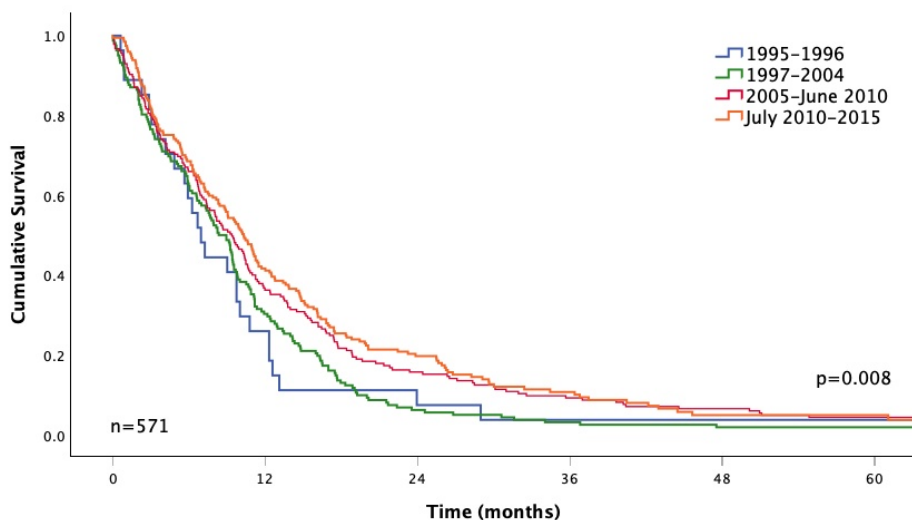


Figure 4 Kaplan-Meier graph illustrating overall survival for 571 patients with glioma, WHO grade 4 diagnosed from 1995–2015 by year of diagnosis. Log-rank test. Modified from Paper I.

We found a stepwise improvement in median OS from 6.9 months (95% CI 5.3–8.6 months) to 10.3 months (95% CI 8.9–11.7 months) (Figure 4) and an increase in 2-year survival from 7% to 18% from the first to the last period.

Treatment and survival in the biobank cohort

The median OS in the biobank cohort was 10.6 months (95% CI 9.6–11.7 months) (Table 5). In the biobank cohort, 76 (31%) patients underwent a tumor resection $\geq 95\%$, 100 (41%) patients had a tumor resection $< 95\%$ and 68 (28%) had a biopsy. Radiochemotherapy was initiated for 134 (55%) patients (Table 5).

In the univariable survival analysis, we found a longer median OS to be associated with young age, good preoperative WHO PS, extensive tumor resection, postoperative radiochemotherapy or treatment within a clinical trial, and absence of cardiovascular disease, diabetes/metabolic disease and inflammatory disease (Table 6). In the multivariable survival analysis, mainly the same factors were associated with a longer median OS: young age, good preoperative WHO PS, absence of diabetes and inflammatory disease, more extensive surgery and postoperative radiochemotherapy or treatment within a clinical trial (Figure 5).

Table 5 Presentation of clinical data for adult patients in the northern Sweden health care region with glioma, WHO grade 4 diagnosed 1995–2015. Data is presented for the following groups: all 571 patients 1995–2015, all 382 patients 2005–2015, the 244 patients in the biobank cohort in Study I, and the patients in the study cohort in Study III; 141 patients with glioblastoma, IDH-wildtype and 9 patients with astrocytoma, IDH-mutant, grade 4.

		All glioma, WHO grade 4 1995–2015 n=571	All glioma, WHO grade 4 2005–2015 n=382	Glioma, WHO grade 4 in biobank cohort Study I n=244	Glioblastoma Study III n=141	Astrocytoma, IDH-mutant, WHO grade 4 Study III n=9
Sex						
No (%)	Male	352 (61.6)	239 (62.6)	155 (63.5)	93 (66.0)	3 (33.3)
	Female	219 (38.4)	143 (37.4)	89 (36.5)	48 (34.0)	6 (66.7)
Age at diagnosis						
Median (range)	years	64.4 (19.0–84.2)	65.1 (21.3–84.2)	64.4 (21.3–84.1)	63.3 (34.6–83.7)	42.1 (25.5–62.4)
No (%)	18–39	22 (3.9)	12 (3.1)	12 (4.9)	5 (3.5)	4 (44.4)
	40–59	187 (32.7)	115 (30.1)	75 (30.7)	50 (35.5)	4 (44.4)
	60–69	178 (31.2)	117 (30.6)	79 (32.4)	45 (31.9)	1 (11.1)
	≥70	184 (32.2)	138 (36.1)	78 (32.0)	41 (29.1)	0 (0.0)
Preoperative WHO PS						
No (%)	0–1			169 (69.3)	111 (78.7)	8 (88.8)
	2			55 (22.5)	24 (17.0)	1 (11.1)
	3–4			20 (8.2)	6 (4.3)	0 (0.0)
Surgery						
No (%)	Biopsy		151 (39.5)	68 (27.9)	21 (14.9)	0 (0.0)
	Resection <95%		128 (33.5)	100 (41.0)	70 (49.6)	4 (44.4)
	Resection ≥95%		95 (24.9)	76 (31.1)	50 (35.5)	5 (55.6)
	Autopsy only		8 (2.1)	0 (0.0)	0 (0.0)	0 (0.0)
5-ALA at surgery						
No (%)	Yes			87 (35.7)	46 (32.6)	2 (22.2)
	No			157 (64.3)	95 (67.4)	7 (77.8)
MGMT promoter methylation status						
No (%)	<10%			61 (25.0)	100 (70.9)	1 (11.1)
	≥10%			24 (9.8)	41 (29.1)	8 (88.9)
	Missing			159 (65.2)	0 (0.0)	0 (0.0)
Postoperative treatment						
No (%)	ITT radio- chemotherapy			134 (54.9)	76 (53.9)	2 (22.2)
	Completed radiochemo- therapy			42 (17.2)	23 (16.3)	2 (22.2)
	Interrupted radiochemo- therapy			92 (37.7)	53 (37.6)	0 (0.0)

	Other treatment			68 (27.9)	47 (33.3)	5 (55.6)
	Trial with experimental drug			19 (7.8)	9 (6.4)	2 (22.2)
	Best supportive care			23 (9.4)	9 (6.4)	0 (0.0)
Treatment at tumor recurrence/progression						
No (%)	Antitumoral treatment ¹			126 (51.6)	79 (56.0)	6 (66.7)
	<i>Surgery</i>			50 (20.5)	33 (23.4)	2 (22.2)
	Best supportive care			115 (47.1)	61 (43.3)	2 (22.2)
	No tumor progression			3 (1.2)	1 (0.71)	1 (11.1)
Time to next intervention (TTNI)						
Median (95% CI)	Months				7.1 (6.1–8.2)	10.2 (1.6–18.8)
Survival						
Median (95% CI)	Months	9.3 (8.5–10.1)	9.8 (8.7–10.9)	10.6 (9.6–11.7)	11.1 (9.6–12.6)	70.6 (0.0–252.2)
	2-year survival (%)	13.3	17.8	20.5	17.7	55.6
	5-year survival (%)	2.8	3.9	3.7	2.8	55.6

¹ Antitumoral treatment includes surgery, radiotherapy and/or chemotherapy/bevacizumab.

During the study period, all except three patients had a tumor progression. At tumor progression, about half of the patients (115 patients (47%)) did not receive any antitumoral treatment. Fifty patients (20%) had a reoperation, in most cases in combination with chemotherapy/bevacizumab, 10 patients (4%) had radiotherapy in combination with chemotherapy/bevacizumab, and 66 patients (27%) had chemotherapy/bevacizumab (Figure 6).

Missing data

In this study, detailed clinical information was available for the biobank cohort but not for the main study cohort. *MGMT* promoter methylation status was only available for a subgroup of the biobank cohort and IDH status was not available. Consequently, data on well-known prognostic factors are missing, to the greatest extent in the main study cohort but also in the biobank cohort.

Table 6 Univariable survival analysis for patients with glioma, WHO grade 4 in the biobank cohort (n=244, except for the analysis for cardiovascular disease with one missing). Log-rank test.

		N	Median overall survival months (95% CI)	p-value
Age at diagnosis				
	<median	122	15.6 (13.2–17.9)	
	≥median	122	8.7 (6.8–10.5)	p<0.001
Preoperative WHO PS				
	WHO PS 0–1	169	14.7 (12.0–17.4)	
	WHO PS 2	55	7.2 (4.9–9.5)	
	WHO PS 3–4	20	3.6 (1.8–5.3)	p<0.001
Extent of surgery				
	Resection ≥95%	76	19.6 (17.4–21.9)	
	Resection <95%	100	11.0 (9.1–13.0)	
	Biopsy	68	5.7 (4.2–7.2)	p<0.001
Postoperative treatment				
	Intention to treat radiochemotherapy	134	15.1 (13.0–17.2)	
	Other treatment	68	8.4 (6.2–10.6)	
	Trial with experimental drug	19	15.8 (14.3–17.3)	
	Best supportive care	23	1.6 (0.6–2.7)	p<0.001
Cardiovascular disease				
	Yes	122	9.0 (7.5–10.6)	
	No	121	13.9 (10.2–16.9)	p<0.001
Diabetes/metabolic disease				
	Yes	39	6.8 (4.2–9.3)	
	No	205	11.4 (9.8–12.9)	p<0.01
Inflammatory disease				
	Yes	27	6.6 (0.6–12.6)	
	No	217	11.2 (9.7–12.6)	p<0.001

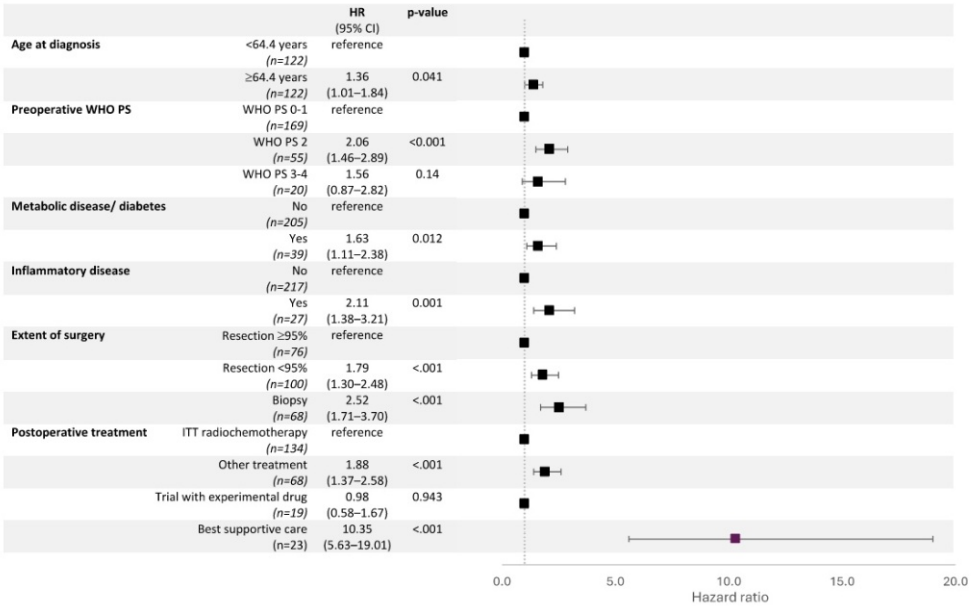


Figure 5 Cox-regression analysis of factors related to overall survival for 244 patients with glioma, WHO grade 4 in the biobank cohort. Modified from Paper I.

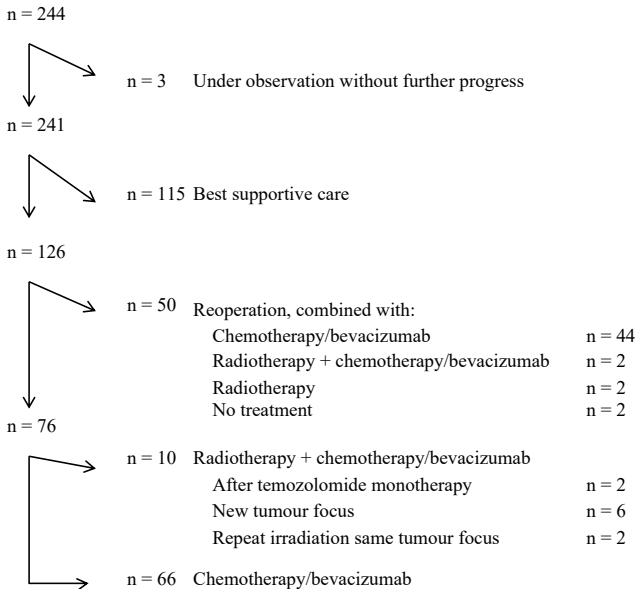


Figure 6 Treatment at tumor progression for 244 patients with glioma, WHO grade 4 in the biobank cohort. Reproduced from Paper I.

Results Study II

Patient characteristics

The study cohort included 1276 patients with glioma, WHO grade 1–4 diagnosed between 2009–2013 and registered in the National Quality Registry for CNS Tumors in the northern Sweden and in the southern regions with high registry coverage. The study cohort included 799 patients with glioma, WHO grade 4, 214 patients with glioma, WHO grade 3, and 263 patients with glioma, WHO grade 1–2. In the analysis, glioma, WHO grade 1–2 was treated as one group (low-grade glioma) and glioma, WHO grade 3–4 as one group (high-grade glioma). Glioma, WHO grade 4 was also studied separately.

Travel time to the regional hospital

As expected because of the geographic differences, patients in northern Sweden had longer travel times to the regional hospital compared to patients in the Southern regions. A longer travel time to the regional hospital was associated with lower educational level for all groups and there was a tendency of an association between longer travel time and older age at diagnosis (Table 7).

Regional comparison

Comparing northern Sweden to the southern regions, patients with glioma, WHO grade 3–4 in northern Sweden were older at diagnosis and more often had less extensive surgery (for example 43% biopsies in northern Sweden and 23% in the Southern regions ($p < 0.001$)). There was a tendency for better WHO PS in the southern regions, but the data were incomplete. The educational level and cohabitation status did not differ between the regions, but the travel times were longer in the North.

Table 7 Mean travel time to the regional hospital in minutes. Differences within each patient group are assessed by *t*-test for region and sex and ANOVA for age categories and education levels. Reproduced from Paper II.

		Glioma, WHO grade 1–2 Mean travel time (n)	Glioma, WHO grade 3–4 Mean travel time (n)	Glioma, WHO grade 4 Mean travel time (n)
Region	Northern Sweden	156.0 (50)	169.5 (209)	172.0 (176)
	Southern regions	52.2 (213)	57.7 (804)	56.6 (623)
	<i>p</i> -value	<0.001	<0.001	<0.001
Sex	Men	75.1 (134)	80.6 (621)	80.1 (490)
	Women	68.6 (129)	81.1 (392)	84.9 (309)
	<i>p</i> -value	0.50	0.92	0.45
Age at diagnosis (years)	0–39	64.9 (96)	64.3 (58)	70.8 (26)
	40–59	65.2 (102)	74.4 (328)	72.4 (251)
	60–69	97.7 (43)	80.5 (341)	81.3 (281)
	≥70	83.2 (22)	91.7 (286)	94.0 (241)
	<i>p</i> -value	0.080	0.042	0.046
Education level	Middle school	102.4 (45)	94.0 (258)	96.1 (211)
	High school	75.5 (106)	85.7 (416)	87.4 (319)
	University/college	55.3 (107)	63.9 (332)	64.2 (264)
	<i>p</i> -value	0.002	<0.001	<0.001

Survival differences in relation to sociodemographic factors

In the multivariable survival analyses for all groups, a longer survival was significantly associated with younger age at diagnosis, but no association was found with sex. Extensive surgery was associated with a survival advantage in glioma, WHO grade 3–4 and glioma, WHO grade 4 (Figure 7).

When studying the sociodemographic covariates for glioma, WHO grade 3–4 patients, survival was significantly longer for patients with higher educational level. For glioma, WHO grade 4 alone, a similar association between survival and educational level was observed but was not statistically significant (Figure 7).

No survival differences were found for travel time to the regional hospital, cohabitation status or region of residence for glioma, WHO grade 3–4 or glioma, WHO grade 4. For glioma, WHO grade 1–2, no associations were found between any of the sociodemographic covariates and survival in the multivariable analysis.

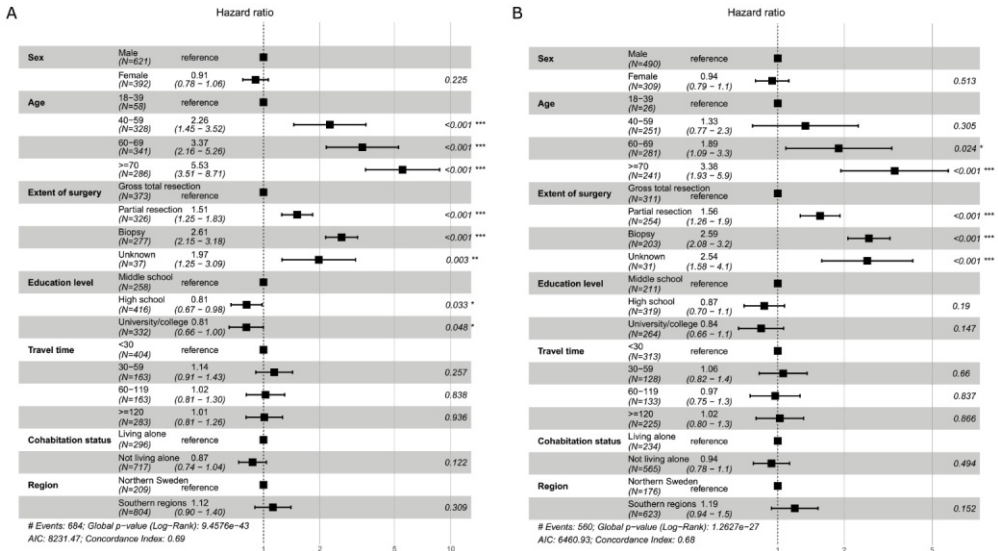


Figure 7 Cox regression analysis of factors associated with overall survival for (A) 1013 patients with glioma, WHO grade 3–4, and (B) 799 patients with glioma, WHO grade 4. Reproduced from Paper II.

Glioma, WHO grade 3–4 was studied in subgroups according to age at diagnosis and a longer survival was demonstrated with higher educational level among the patients aged 40–49 years at diagnosis (middle school (ref) HR: 1, high school HR: 0.60, CI [0.41–0.88], university/college HR: 0.63 CI [0.43–0.93], $p=0.023$). In the other age groups, no differences in survival related to education were found.

Missing data

Data on WHO PS and smoking were incomplete with approximately 30% missing data, and those variables were therefore not included in further analysis. *MGMT* promoter methylation status and IDH status were not available. The missing data for WHO PS, *MGMT* promoter methylation status, and IDH status is a limitation in the study since they all have prognostic value. Smoking status has an association with SES and comorbidities and would have been a valuable confounder.

Results Study III

Patient characteristics

The study included 150 patients diagnosed with a glioblastoma or an astrocytoma, IDH-mutant, grade 4 between 2005–2015 and with a tumor tissue sample from primary surgery in the biobank. A large majority of the study cohort, 141 patients had a glioblastoma and 9 patients an astrocytoma, IDH-mutant, grade 4.

Compared to all patients in northern Sweden diagnosed during the same years, the patients in the study cohort were younger (median age at diagnosis 63.3 years (range 34.6–83.7) for glioblastoma and 42.1 years (range 25.5–62.4) for astrocytoma, IDH-mutant, grade 4 vs 65.1 years (range 21.3–84.2) for all patients) and had a longer median OS (11.1 months (95% CI 9.6–12.6) for glioblastoma and 70.6 months (95% CI 0.0–252.2) for astrocytoma, IDH-mutant, grade 4 vs 9.8 months (95% CI 8.7–10.9) for all patients) (Table 5).

Compared to glioblastoma, the patients with an astrocytoma, IDH-mutant, grade 4 were younger at diagnosis (median 42.1 years (range 25.5–62.4) vs 63.3 years (range 34.6–83.7), $p < 0.001$), more often had a *MGMT* promoter methylation (89% vs 29%, $p < 0.001$), and had a longer median OS (70.6 months (95% CI 0.0–252.2) vs 11.1 months (95% CI 9.6–12.6), $p = 0.001$) (Table 5). There were no significant differences between the IDH-mutant and IDH-wildtype groups according to the frequency of comorbidities, smoking, preoperative WHO PS, the extent of surgery, the use of 5-ALA at surgery, postoperative treatment or TTNI.

Predictive biomarkers were studied in 68 patients with glioblastoma treated with resecting surgery and planned for radiochemotherapy (the ITT radiochemotherapy group). These patients had a median age at diagnosis of 61.7 years (range 37.0–76.4), a WHO PS of most often 0–1 (85% of the patients) but in some cases 2 (15%) and had a median OS of 15.5 months (95% CI 13.4–17.3 months).

Metabolite analysis in glioblastoma

In the metabolite analysis for OS, ten metabolites were significantly associated with prognosis in the Cox proportional hazard analysis. High concentrations of sorbitol-6-phosphate, pentadecanoic acid, s-adenosyl-L-methionine (SAM), 1-eicosapentaenoyl GPE, cysteine, a mix of 5-carbon sugar alcohols (xylitol/arabitol/ribitol), myo-inositol, epi-

inositol, scyllo-inositol and low concentration of 2-methylglutaric acid were associated with a longer OS.

In the metabolite analysis for TTNI high concentrations of alpha-ketoglutaric acid, glycerol-2-phosphate, homoarginine, arginine, and xylitol/arabitol/ribitol, and low concentration of cellobiose/laminaribiose/maltose were associated with a longer TTNI.

Metabolite analysis in glioblastoma subgroups

The metabolite analyses were performed in subgroups of glioblastoma according to preoperative tumor volume, *MGMT* promoter methylation status and postoperative treatment. In large tumors, amino acids, fatty acids, acylcarnitines and saccharides were associated with OS and TTNI, In the small tumors we found sugar alcohols and metabolites involved in the polyol pathway. In the *MGMT* promoter methylated tumors a more metabolically active profile was found compared to the unmethylated tumors. For *MGMT* promoter methylated tumors, the analyses highlighted amino acid metabolites, fatty acids and acylcarnitines and in the unmethylated, carbohydrates and lipid metabolites. The results of the metabolite analyses in glioblastoma subgroups are described in detail in Paper III.

Predictive metabolic markers in glioblastoma

To study predictive biomarkers for treatment with resective surgery and postoperative radiotherapy, TTNI for patients in the ITT radiochemotherapy group who underwent resective surgery was studied. We found high concentration of indolelactate, and low concentrations of uridine 5'-diphospho-N-acetylglucosamine (UDP-Glc-NAc) and 5,6-dihydrouracil to be associated with a longer median TTNI.

Prognostic and predictive metabolite scores in glioblastoma

Metabolite scores were created through combining the information from the concentrations of the ten significant metabolites from the OS analysis for glioblastoma or the three significant metabolites from the TTNI analysis for glioblastoma patients with resective surgery in the ITT radiochemotherapy group.

The prognostic and predictive metabolite scores were associated with OS or TTNI both in the univariable and in the multivariable survival analysis. In the multivariable analysis for the prognostic score, survival was significantly shorter for patients with older age at diagnosis (18–59 years (ref) HR: 1, 60–69 years HR: 1.43 CI [0.78–2.62] p=0.24, >70

years HR: 2.23 CI [1.27–3.88] $p=0.005$), less extensive surgery (resection (ref) HR: 1, biopsy HR: 3.09 CI [1.53–6.22] $p=0.002$) and low metabolite score (high (ref) HR: 1, low HR: 1.78 CI [1.11–2.83] $p=0.02$). In the same analysis, no significant associations were found between survival and sex, *MGMT* promoter methylation status or preoperative WHO PS. For the predictive score, TTNI was significantly shorter for patients with low metabolite score (high (ref) HR: 1, low HR: 3.07 CI [1.27–7.41] $p=0.01$) but no associations were found with sex, age at diagnosis. *MGMT* promoter methylation status or preoperative WHO PS.

Metabolite analysis in astrocytoma, IDH-mutant, grade 4

In the small group of IDH-mutant tumors ($n=9$), different metabolites were associated with OS and TTNI compared to glioblastoma. Mainly amino acids and saccharides were found in the OS analysis and for TTNI medium and long-chain acylcarnitines. The results are shown in detail in Paper III.

Congruency between OS and TTNI

The correlation between OS and TTNI was studied for the individual patients. For glioblastoma there was a quite clear association between OS and TTNI in the correlation analysis ($R^2=0.88$), but no correlation was found in the astrocytoma, IDH-mutant, grade 4 group ($R^2=0.046$). We also studied the congruency between the metabolites associated with OS and TTNI respectively and found that most metabolites were related to OS or TTNI but not with both.

Discussion

Main findings in relation to aims

This thesis studies different prognostic and predictive factors for glioma. We have described the study cohorts with aspect to baseline characteristics and treatment and analyzed survival in relation to tumor-related, treatment-related and patient-related factors. Through these studies we have enhanced the knowledge on prognostic and predictive factors for glioma.

The changes over time regarding treatment and survival for patients with glioma, WHO grade 4 has been described in Paper I. The survival has increased, both with an increase in median OS and 2-year survival. During the studied period, a new WHO classification was implemented, and postoperative radiochemotherapy and the use of 5-ALA at surgery were introduced. These changes are suggested to contribute to the reported increased survival.

The possible association between glioma patients' survival and sociodemographic factors was studied in Paper II. A low educational level was related to a shorter median OS for patients with glioma, WHO grade 3–4. The other studied sociodemographic factors were not associated with prognosis for patients with glioma, WHO grade 1–4.

In Paper III metabolite analyses were performed to study prognostic and predictive metabolic markers for glioblastoma and astrocytoma, IDH-mutant, grade 4. In this study we validated earlier results on prognostic metabolic markers in glioblastoma and presented new results regarding prognostic metabolic markers in glioblastoma subgroups and predictive metabolic markers. We could also combine the prognostic and predictive metabolites into metabolite scores with prognostic and predictive information in an effort to create a future clinical meaningful measure.

Methodological considerations

Study size

The study size varies in the different studies in this thesis, from the RISK North cohort with 1276 included patients to the study cohort in Study III including 150 patients. Glioma, WHO grade 4 is, even though it is the most common glioma, not a very common disease and the population in

northern Sweden is limited. A rare disease in combination with a limited population makes it difficult to recruit large study cohorts. This is a considerably larger problem when studying astrocytoma, IDH-mutant, grade 4 and subgroups of glioblastoma. A small sample size limits the statistical power, decreases the possibility to detect true differences statistically and increases the risk of false positive results ¹²⁷. Analyses in small samples should therefore be interpreted with caution.

Glioma classification

To study a cohort diagnosed over a long time during which diagnostic procedures and classifications have changed might influence the analysis and results. Changes in classifications during the study period raises the question of whether the study cohort consist of one tumor entity or if patients with slightly different tumors are included over time. The classification of brain tumors can also be a challenge, not only because of the many different brain tumors. In addition, the tumors are not always distinct biologic entities but there could be a successive change between tumor types that makes the classification difficult. There is a possibility to classify a tumor that does not fit into a distinct entity as not elsewhere classified (NEC) ². In those cases, a descriptive diagnosis is made by the pathologist.

In Study I the difficulties in defining the study cohort is obvious with the shift between grade 3 and grade 4 tumors from the 1st to the 2nd edition of the WHO classification that resulted in a higher incidence of grade 4 tumors and consequently, a lower incidence of grade 3 tumors. In Study III the classification difficulties were addressed through reclassification of the tumors according to the revised 4th edition of the WHO classification from 2016. In Study II, the diagnosis reported in the National Quality Registry for CNS tumors was the basis for patient selection, but during the whole study period (2009–2013) the same classification was used.

Data quality

The collection of clinical data may be difficult, especially when registering data retrospectively. In the studies that this thesis is based on, different methods were used for achieving clinical data. For Studies I and III, clinical data were retrospectively collected from medical records. Data in medical records are not always complete due to differences in record keeping. For example, WHO PS was not always documented but was for the studies registered for all patients through an estimation from the available documentation in the medical records. Today, registering

WHO PS is, at some centra, mandatory at each contact with health care. This will facilitate future research since WHO PS is a strong and well documented prognostic factor for many diseases ^{128,129}. The estimation of WHO PS is, even in prospective registration, to an extent subjective and there could be individual differences between physicians. For Studies I and III, the clinical data is crucial for the analyses and, despite the difficulties in retrospectively collecting data from medical records, this is the only possible way of achieving the clinical data. To minimize possible inaccuracies of incorrect retrospective registration, a superior method would have been to prospectively collect clinical data.

For Study II, data were obtained from the RISK North. When retrieving data from a registry we are dependent on correct registration of data and a good registry coverage. The Swedish registries and the possibility to link data through the unique personal identity number provides great opportunities for research. The linking of data enables the use of different health care and demographic registries to study possible associations between data from different sources.

Some quality registries are evaluated for data quality, for example completeness of data, through comparing registry data with data from medical records ¹³⁰. The National Quality Registry for CNS Tumors is evaluated for coverage by the Regional Cancer Centers in Sweden through comparison with the National Cancer Registry, and evaluation for data validity is currently ongoing.

In Study II, data on WHO PS and smoking status were incomplete and were therefore not included in the analysis and information on comorbidities was not available. As described earlier, WHO PS is an important prognostic factor for glioma patients. Smoking is associated with SES, with a higher proportion of smokers among persons with lower education and income ¹³¹ and smoking is associated with several diseases, e.g. cardiovascular and pulmonary diseases. In the analysis of the association between SES and prognosis, the inability to adjust for WHO PS and smoking, and the lack of information on comorbidities is a limitation.

Analysis of metabolite data

In Study III the number of analyses is very large with a following risk of false positive results. To reduce this risk, a HR of <0.91 or >1.10 was required in addition to a p-value <0.05 in the Cox proportional hazard analysis. Another option would be to use some kind of correction for

multiple testing or the use of separate cohorts for primary analysis and validation. In the initial data processing, two cohorts were used for testing and validation but in further analyses we chose to use the whole cohort in the primary analysis to obtain maximal power in the analysis. There are also other possible methods for analyzing the metabolite data such as multivariate models searching for patterns in the metabolite data.

General discussion

Studying prognostic factors is challenging partly because the patient's survival is affected by multiple factors. Our studies reveal associations between survival and different prognostic factors, but causality is not possible to prove. The impact of different prognostic factors is partly addressed through multivariable analyses with adjustment for potential confounders.

Survival for patients with glioma, WHO grade 4 in northern Sweden

In Paper I we study real world data to evaluate if the results from clinical trials can be translated into the daily clinical work. We found a small, stepwise improvement in OS over time which is similar to previous studies^{68,132,133}. In the multivariable analysis, both the extent of surgical resection and postoperative treatment were associated with prognosis despite adjusting for age, WHO PS and comorbidities. Earlier studies have described the same prognostic factors in glioma, WHO grade 4; age, WHO PS, extensive tumor resection and postoperative treatment^{10,64,65,67,68,134}.

During the study period, three changes in diagnostic and treatment routines were described: a new WHO classification with a shift from WHO grade 3 tumors to WHO grade 4, the implementation of postoperative radiochemotherapy, and the use of 5-ALA at resective surgery in clinical routine. These changes could be one reason for the improvement in survival that was observed in the study. Other possible explanations could be earlier diagnosis through a larger use of neuroradiology, earlier health care seeking from the patients or treatment at tumor progression. The present thesis did not address how the patients were diagnosed with glioma and as a result, potential temporal differences in diagnostic pathways or patterns of healthcare-seeking behavior could not be evaluated. So far, the evidence for an effect on survival of treatment at tumor progression is limited and

patients that are eligible for antitumoral treatment at tumor progression are often those with other positive prognostic factors, e.g. good WHO PS and young age. Therefore, it is difficult to assess the specific effect of the treatment in retrospective studies.

Evaluating new treatments in clinical routine is important since they usually are based on studies that include a selected group of patients in good WHO PS and without major comorbidities. Studying a treatment in the daily clinical setting provides information of the effect and benefit of the treatment in a less selected patient cohort, and the frequency of potential rare side effects that not always turn up in the limited cohort in a clinical trial. Even though the biobank cohort is more representative from the daily clinical perspective than the cohorts in clinical trials, there are differences for example regarding extent of surgery between the biobank cohort and all glioma, WHO grade 4 patients diagnosed in the region during the same time. One possible explanation for the lower proportion of patients undergoing a biopsy in the biobank cohort is that the amount of tumor tissue from a biopsy could be too sparse to allow both diagnostic analyses and storing in the biobank. To fully evaluate a treatment in clinical routine, we need complete, population-based data regarding diagnosis, clinical and tumor-related prognostic factors, and treatment. This kind of data might in the future be retrieved from the National Quality Registers if the coverage is good and sufficient data registered. For Studies I and III, the National Quality Registry for CNS Tumors could not be used since the registry was not validated and the registration incomplete.

Sociodemographic factors and differences in glioma prognosis

In Paper II, we highlight glioma prognosis from another point of view through studying sociodemographic factors. Sociodemographic factors might affect the outcome for glioma patients, and these studies are important for finding potential differences that can be possible to address and manage to reach health equity. In Study II, the association between prognosis and educational level, travel time to the regional hospital, cohabitation status and region of residence was studied. Educational level was the only of the studied sociodemographic covariates that was associated with outcome with a longer median OS for glioma, WHO grade 3–4 patients with a higher educational level.

In Sweden, the treatment and care for glioma should be evidence-based and offered in an equal manner to all patients regardless of

sociodemographic differences. Only a minority of the costs for the health care are covered by the patient fees and an individual health insurance is not needed for access to the public health care. The association between educational level and prognosis could be affected by other prognostic factors or differences in the access to health care. In our study, the association between educational level and survival was found despite adjusting for sex, age at diagnosis, extent of surgery, travel time to the regional hospital and region of residence and hence we have adjusted for several important prognostic factors. Information on additional prognostic factors, including treatment modalities beyond type of surgery and tumor-related prognostic factors (e.g. IDH status and *MGMT* promoter methylation) were not available and could have contributed to the observed prognostic differences.

The access to health care can be related to several factors, for example the availability of health care both regarding resources, organization, geography, and financial aspects ^{135,136}. For a good access to health care, the patient needs knowledge about how to have their needs met, have the ability to physically reach the health care facility and to be able and willing to pay possible charges. The health care provider must have the necessary resources, both educated staff and technical equipment. Previous studies indicate differences in access to health care between urban and rural areas ¹³⁷ and an association between higher SES and lower preoperative tumor volumes possibly related to differences in health care seeking behavior ⁹⁰. In Study II, the access to health care is not studied and the possible impact of these factors cannot be evaluated.

The association between educational level and survival was only demonstrated in a quite young group of patients with glioma, WHO grade 3–4. In this age-group in Sweden, it is uncommon to have an education of nine years or less and we suspect that there are other correlating factors involved in this association. In Sweden in 2024, approximately 10% of the population between 25–64 years had a highest completed educational level of nine years or less, and low educational level was more common among men, older persons and individuals born outside Sweden ¹⁰⁰. In general, the educational attainment is increasing over time in Sweden ¹⁰⁰ and consequently SES in the population varies over time. The educational level in the population over the short study period is not expected to change and the differences in education between older and younger patients should partly be addressed by adjusting for age at diagnosis. Previous research has described the educational attainment to be associated with both cognitive abilities early in life, parental resources and social support ¹³⁸. A possible

explanation that we cannot evaluate in Study II could be differences in comorbidities or cognitive abilities affecting both the educational level and survival if the person is diagnosed with a glioma. Cognitive abilities early in life and certain comorbidities might influence both the individual's educational attainment and the treatment possibilities at glioma diagnosis. Further studies are needed to understand the association between educational level and survival in this group of patients.

The other sociodemographic factors, travel time to the regional hospital, cohabitation status and region of residence were not associated with survival in any group. The lack of an association between travel time to the regional hospital and survival does not indicate undertreatment regarding surgery, radiotherapy and/or medical oncological treatment for patients with long travel time. Medical oncological treatment is often possible for the patient to receive at local hospitals closer to their home even though the neuro-oncologists are working at the regional hospitals. The patients with long travel time could also benefit from a good help from primary care or local hospitals in treating for example infections or thromboses.

Patients with brain tumors commonly have cognitive impairments and often need support from other persons. Studying the role of close relatives might therefore be especially important for those patients. The support provided by the relative could be both mental, physical and economic ⁹³. In Sweden, living together without being married is common and those patients should benefit from the same support from their cohabitant as married patients. When only studying marriage, there is a possibility to underestimate the effect of cohabitation status. In Study II, cohabitation status was not associated with survival. One potential explanation might be that the lack of support from the cohabitant is at least partly compensated by someone else – for example other relatives or support from the municipalities or the health care if needed.

When comparing northern Sweden with the Southern regions, we found no differences in survival despite the patients in northern Sweden being older, more often have less extensive surgery and have a tendency of a worse WHO PS. Taken together, these negative prognostic factors should theoretically be related to a worse survival, but this was not the case in our study. This is not easy to explain from the available data. The probability of incorrect data is most likely low regarding age and type of surgery but assessing WHO PS could be more diverging.

Prognostic and predictive metabolic markers for glioma, WHO grade 4

In Paper III, we present results from the metabolite analysis on tumor tissue samples from primary surgery. We report significant metabolic markers with prognostic information for glioblastoma and predictive metabolic markers in the group treated with resective surgery and radiochemotherapy. The significant metabolites were combined to calculate scores with prognostic and predictive information for glioblastoma. Metabolic markers in glioblastoma subgroups according to preoperative tumor volume, *MGMT* promoter methylation status and treatment, and for astrocytoma, IDH-mutant, grade 4 were also presented.

The prognostic metabolites in glioblastoma are mainly related to antioxidant and redox capacity and are largely reported from previous studies. Tumor cells often depend more on antioxidants compared to normal tissue, and antioxidants may both promote and inhibit tumor progression in different contexts, in glioma redox imbalance is described as a core vulnerability¹³⁹. In Paper III, the significant prognostic metabolites include sorbitol-6-phosphate, SAM, cysteine, 5-carbon sugar alcohols and myo-inositol and its stereoisomers. Previous studies have demonstrated antitumoral effects of SAM on glioblastoma cell lines *in vitro*¹⁴⁰, cysteine as a factor limiting proliferation and survival of glioma cells¹⁴¹, 5-carbon sugar alcohols to correlate with malignancy and to be associated with survival for glioblastoma^{110,112}, and myo-inositol have been associated with survival^{110,116} and tumor aggressiveness^{112,142}.

In the analysis in glioblastoma subgroups, the *MGMT* promoter methylated tumors differ from the tumors with unmethylated *MGMT* promoter with a more metabolically active profile in the methylated tumors. In the *MGMT* promoter methylated group, mainly metabolites associated with amino acid metabolism and antioxidants were associated with OS and TTNI. In the *MGMT* promoter unmethylated group, we found instead metabolites involved in carbohydrate and lipid metabolism. The *MGMT* methylated and unmethylated tumors generally differ in treatment response and prognosis, and those metabolic differences might reflect the partly different clinical behavior of these tumors.

A hypoxic metabolic environment is frequent in gliomas and affects the metabolism in tumor cells¹¹⁸, and metabolic changes to enable cell growth under anabolic and nutrient deprived conditions are common¹⁰⁵.

One possible explanation to metabolic differences between large and small tumors could be a larger extent of hypoxic areas and necrosis in large tumors. In hypoxic conditions, changes are observed in pathways both for energy production and biosynthesis of raw materials ¹⁴³.

The analysis of predictive metabolic markers for surgery and radiochemotherapy identified three significant metabolites associated with metabolic pathways linked to tumor growth: indolelactate, 5,6-dihydrouracil and UDP-Glc-NAc. Indolelactate is involved in tryptophane metabolism and has been implicated in immune evasion and tumor progression ^{144,145}. Previous studies have also associated tryptophane metabolites with prognosis and treatment response in different cancers including breast cancer and gastric cancer ¹⁴⁶. UDP-Glc-NAc is generated through the hexosamine biosynthetic pathway and plays a role in transcriptional regulation, cellular proliferation, and apoptosis. Dysregulation of this pathway has been demonstrated in cancer cells and has been linked to enhanced tumor growth ^{147,148}. 5,6-dihydrouracil, a metabolite of uracil, has previously been associated with tumor aggressiveness and elevated levels have been detected in microdialysate from high-grade glioma compared to adjacent brain tissue during interstitial cisplatin treatment ¹¹².

The metabolite analysis in the small group of astrocytoma, IDH-mutant, grade 4 tumors indicate reduced biosynthetic and oxidative activity, and this less proliferative profile might reflect the less aggressive clinical behavior of these tumors. The metabolites associated with OS and TTNI in this group were different from the metabolites found in the analysis in glioblastoma which supports the previous studies showing metabolic differences between subtypes of glioma ¹⁰⁹.

In the analyses, for all groups, there was in general a small overlap between the metabolites associated with OS and TTNI. This could indicate that different metabolic mechanisms are involved when studying survival and treatment response. When analyzing the correlation between OS and TTNI for the patients, a quite strong correlation was found in glioblastoma but not in astrocytoma, IDH-mutant, grade 4. The survival for astrocytoma, IDH-mutant, grade 4 was considerably longer than for glioblastoma (70.6 months vs 11.1 months), but the differences in TTNI were less pronounced (10.2 months vs 7.1 months). These differences in metabolites and between OS and TTNI implicates that other factors than postoperative treatment affect survival, for example biological differences between the tumors. Antitumoral treatment after tumor progression have so far shown

limited effects and cannot be expected to have a large impact on survival^{15,16}.

The prognostic and predictive metabolite scores created through merging the significant prognostic and predictive metabolites respectively show an association with OS and TTNI in multivariable models adjusted for other known prognostic factors. These results indicate that it is possible to use information from metabolite data to obtain a prognostic or predictive tool. Further studies are needed to develop metabolite scores that are possible to use in clinical routine. Validated prognostic and predictive scores might be useful in treatment decisions, and predictive scores especially in the choice of treatment for the individual patient.

Our findings regarding prognostic biomarkers are compared to earlier published results, but the results of the subgroup analyses, predictive biomarkers and the metabolite scores need validation in future studies in other cohorts.

The metabolite analyses in Paper III were performed on frozen tumor tissue. For the patients with a tumor tissue sample in the biobank, blood samples were also collected at surgery and during further treatment. Obviously, blood samples are easier to obtain, and repeated samples are possible for treatment evaluation or detection of tumor progression or recurrence. In blood samples, a larger impact from exogenous substances is expected compared to tumor tissue. Previous studies investigating serum samples and tumor tissue or microdialysate from tumor, have found similar differences between glioma subtypes and diagnostic response patterns during treatment in both serum and tumor which indicates systemic changes^{110,149}. These results implicate that analyses on blood samples could be valuable in addition to analyses on tumor tissue.

Conclusions

This thesis provides additional insight into factors related to survival and treatment response in glioma. Survival has improved over time, potentially reflecting advances in treatment and care. Among young patients with glioma, WHO grade 3–4, higher educational level was associated with longer survival, while the other studied sociodemographic factors were not related to outcome in the Swedish health care setting. Metabolite analyses identified metabolic markers associated with survival and treatment response, which were combined into metabolite-based scores with prognostic and predictive relevance in glioblastoma.

Future perspectives

Despite the progress made in treatment of glioblastoma, IDH-wildtype and astrocytoma, IDH-mutant, grade 4 the prognosis is still dismal, and the evidence-based treatment options are limited. Several clinical trials evaluating novel treatments have yielded negative results or demonstrated only a limited effect. There is a substantial need for future continued research to improve the understanding of mechanisms underlying treatment resistance and with the aim of developing more effective treatments both at time for diagnosis and at tumor progression.

Continued studies on metabolite analyses of blood samples from the biobank is an important next step in the project described in this thesis. A possibility of using metabolite analyses of blood samples in the choice of treatment, in treatment evaluation and for earlier detection of tumor progression in the future would be useful in the clinical setting.

The treatment with TTFields is a relatively recent addition to clinical practice and was implemented after the collection of tissue samples and clinical data used in the projects included in this thesis. The treatment is already planned to be evaluated using real-world data from the National Quality Registry for CNS tumors in Sweden and metabolite analyses performed during TTFields treatment may provide further insights.

Another important area of future research is studies based on the patient's perspective. When introducing new treatments, qualitative studies of the patient's experience of the treatment and potential side effects are necessary in addition to assessing the effect of the treatment on the disease.

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