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*Original Article*

# A population-based study of high-grade gliomas and mutated isocitrate dehydrogenase 1

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**Abstract:** High-grade gliomas have a dismal prognosis, and prognostic factors are needed to optimize treatment algorithms. In this study we identified clinical prognostic factors as well as the prognostic value of isocitrate dehydrogenase 1 (IDH1) status in a population-based group of patients with high-grade gliomas. Using the Danish Cancer Registry and the Danish Pathology Databank we identified 359 patients: 234 had WHO grade IV gliomas, 58 had WHO grade III gliomas, and 67 were diagnosed clinically. Mutated IDH1 was predominantly observed in oligodendroglial tumors (WHO grade III). Patients with mutated IDH1 had a significantly better outcome than patients with wildtype IDH1: 2-year OS 59% and 18%, respectively (HR 0.38, 95% CI 0.21-0.68). However, when adjusting for other prognostic factors, IDH1 status was not a significant independent prognostic factor (HR=0.58, 95% CI 0.32-1.07). Young age, absence of neurological deficit, performance status 0–1, tumor not crossing the midline, and receiving post-surgical treatment were significant independent indicators of a good prognosis in multivariate analysis. In conclusion: This population-based study could not demonstrate IDH1 status to be an independent prognostic factor in high-grade gliomas when adjusting for the effect of classic prognostic factors.

**Keywords:** Glioblastoma, high-grade gliomas, prognosis, population characteristics, isocitrate dehydrogenase 1 (IDH1)

## Introduction

The majority of previously published prognostic studies include only patients with a histological verified glioma, and inclusion is limited to younger patients in good performance status and without comorbidity. In daily clinical practice, the percentage of older patients with considerable comorbidity is increasing as is the demand for treatment of these patients, although whether these patients would benefit from treatment is not known. The need for clinical parameters and comprehensive assessment scales that can identify patients who will benefit from treatment becomes more and more apparent, but the numbers of population-based studies are limited [1, 2].

Recently, Lawrence et al. [1] published a large population-based study, and concluded that only patients younger than 70 years in good performance status benefit from treatment. They investigated 13,003 patients with a glioblastoma multiforme (GBM) diagnosed between 2001 and 2007, and reported that age less than 70 years, use of radiation therapy, gross total resection, and a high income are associated with better survival. This result is in agreement with a phase 3 study conducted by The European Organization for Research and Treatment of Cancer (EORTC), Brain Tumor and Radiotherapy Groups, and the National Cancer Institute of Canada (NCIC) Clinical Trials Group in 2005 [3]. The study was based on patients younger than 70 years in good performance status, and the authors showed that the addition of concomitant and adjuvant temozolomide (TZM) to postsurgical radiotherapy increased 2-year survival from 10% to 26% and 5-year survival from 2% to 10% in patients with GBM [4]. In the last decade, several studies on the treatment of older patients have been conducted [5-8], but also these studies include only

highly selected populations. With the growing fragile elderly population demanding treatment, an evaluation of prognostic factors in the complete population of patients is needed.

The purpose of this retrospective study was to describe the complete population of patients with high-grade gliomas (HGGs) in a pre-specified geographical area and in a pre-specified time period. In addition, we sought to identify clinical prognostic factors in this population and investigate the expression and prognostic value of isocitrate dehydrogenase 1 (IDH1).

## Materials and methods

### *Patients*

We retrospectively registered patients with primary HGGs diagnosed during the period 1 January 2005 to 31 December 2009. We included adult residents in the Region of Southern Denmark with anaplastic astrocytomas, anaplastic oligodendroglial tumors, and GBMs.

In the Danish Cancer Registry, 535 patients were identified. The medical and pathological reports for patients with the following ICD-10 codes D33.0-33.2, D33.7-33.9, D43.0-43.2, D43.7-43.9, C71.0-71.9, and C72.8-72.9 were considered for inclusion. We excluded 220 patients, leaving 315 patients for inclusion.

In the Danish Pathology Databank, 397 patients were identified. Patients with the following histological diagnosis were included: M 93813 (gliomatosis cerebri), M 93853 (anaplastic oligo-astrocytoma), M 94013 (anaplastic astrocytoma), M 94403 (glioblastoma multiforme), M 94423 (glio-sarcoma), and M 94513 (anaplastic oligodendroglioma). We excluded 105 patients, leaving 292 patients for inclusion. In addition, we identified and reviewed 437 patients treated at Odense University Hospital in the departments of neurosurgery, neurology, and oncology; 267 of these patients were also included in the study.

A total of 359 patients were included in the survival analysis, of these 218 patients (61%) were registered in all three registers. Sixty-seven patients (19%) had a non-histologically verified diagnosis, the diagnosis being based on radiological scans and the clinical evaluation of a neurosurgeon or a neurologist.

When tissue samples were reviewed, 15 patients were excluded because of inferior tissue material. Patients with no histological verified diagnosis were excluded as well, leaving 277 tissue samples for immunohistochemical analysis.

The following parameters were registered using medical records: primary symptoms (seizures, headache, neurological deficit, and mental disturbance), date of surgery, extent of resection, performance status (PS), and postsurgical treatment. All accessible scans were reviewed, and using the last scan before surgery, the following parameters were registered: date of scan, MRI or CT scan, contrast enhancement, largest tumor diameter and orthogonal diameter, localization, midline shift, and tumor crossing midline. These parameters were chosen because they have been reported as having a prognostic significance in gliomas. If no scan was accessible, data were registered based on the radiological and medical records.

Age groups were defined as follows: younger patients < 60 years of age, middle-aged patients 60–70 years of age, and older patients > 70 years of age. In addition, patients were divided into four groups according to the treatment received. Group I received curative intended treatment. Curative treatment was surgery and high-dose radiotherapy for WHO grade III tumors and surgery, high-dose radiotherapy, and concomitant and adjuvant chemotherapy for WHO grade IV tumors. Group II received palliative treatment consisting of surgery and short-course radiotherapy (34Gy/10 fractions) or chemotherapy. Group III received surgery only, and group IV received supportive care.

The study was approved by the local Committee on Health Research Ethics and the Danish Data Protection Agency. Use of the tissue was not prohibited in any of the patients according to the Danish Tissue Application Register.

### *End-point*

Patients were followed until death; patients still alive were censored on 1 February 2012. Overall survival (OS) was defined as time from primary surgery until death or date of censoring. Recurrence was defined as recurrence on MRI, clinical progression or death. Progression

**Table 1.** Patient characteristics, 2-year overall survival and univariate analyses

| | All | | | WHO grade III | | WHO grade IV | |
|---|---|---|---|---|---|---|---|
| Variable | No (%) | 2-y | HR (95% CI) | No (%) | HR (95% CI) | No (%) | HR (95% CI) |
| All | 359 (100) | 18 | | 58 (100) | | 234 (100) | |
| Gender | | | | | | | |
| Female | 152 (42) | 20 | 1.00 | 21 (36) | 1.00 | 97 (42) | 1.00 |
| Men | 207 (58 | 6 | 1.01 (0.81-1.26) | 37 (64) | 1.08 (0.85-1.37) | 137 (58) | 1.21 (0.92-1.60) |
| Age (years) | | | | | | | |
| <60 | 116 (32) | 35 | 1.00 | 28 (48) | 1.00 | 85 (36) | 1.00 |
| 60-70 | 105 (29) | 4 | 1.96 (*1.47-2-60*) | 21 (36) | 1.59 (*1.16-2.18*) | 77 (33) | 1.58 (*1.14-2.18*) |
| >70 | 138 (39) | 6 | 3.26 (*2.46-4.32*) | 9 (16) | 2.86 (*2.12-3.87*) | 72 (31) | 2.13 (*1.51-2.99*) |
| Seizures | | | | | | | |
| Absent | 284 (79) | 16 | 1.00 | 42 (73) | 1.00 | 183 (78) | 1.00 |
| Present | 75 (21) | 25 | 0.65 (*0.49-0.85*) | 16 (27) | 0.39 (*0.19-0.79*) | 51 (22) | 0.86 (0.62-1.19) |
| Neurologic deficit | | | | | | | |
| Absent | 189 (53) | 22 | 1.00 | 29 (50) | 1.00 | 127 (54) | 1.00 |
| Present | 170 (47) | 13 | 1.31 (*1.06-1.63*) | 29 (50) | 1.58 (0.89-2.81) | 107 (46) | 1.23 (0.94-1.61) |
| Headache | | | | | | | |
| Absent | 283 (79) | 15 | 1.00 | 46 (79) | 1.00 | 175 (75) | 1.00 |
| Present | 76 (21) | 28 | 0.67 (*0.51-0.87*) | 12 (21) | 0.60 (0.29-1.25) | 59 (25) | 0.76 (0.56-1.04) |
| Mental disturbance | | | | | | | |
| Absent | 236 (66) | 21 | 1.00 | 41 (71) | 1.00 | 164 (70) | 1.00 |
| Present | 123 (34) | 11 | 1.57 (*1.25-1.97*) | 17 (29) | 1.59 (0.86-2.93) | 70 (30) | 1.43 (*1.06-1.91*) |
| Performance status | | | | | | | |
| 0-1 | 188 (52) | 31 | 1.00 | 37 (64) | 1.00 | 146 (63) | 1.00 |
| 2-4 | 171 (48) | 3 | 1.58 (*1.47-1.71*) | 21 (26) | 1.53 (*1.26-1.87*) | 88 (37) | 1.59 (*1.44-1.75*) |
| Tumour crossing midline* | | | | | | | |
| No | 285 (79) | 20 | 1.00 | 47 (84) | 1.00 | 196 (84) | 1.00 |
| Yes | 65 (18) | 6 | 1.63 (*1.23-2.15*) | 9 (16) | 1.16 (0.54-2.50) | 36 (15) | 1.73 (*1.20-2.50*) |
| Location | | | | | | | |
| Frontal | 109 (30) | 22 | 1.00 | 23 (40) | 1.00 | 69 (30) | 1.00 |
| Other | 250 (70) | 16 | 1.30 (*1.03-1.66*) | 35 (60) | 1.96 (*1.07-3.59*) | 165 (70) | 1.03 (0.77-1.38) |
| Extent of surgery | | | | | | | |
| Biopsy | 70 (20) | 14 | 1.00 | 32 (55) | 1.00 | 38 (16) | 1.00 |
| Partial | 144 (40) | 23 | 0.74 (*0.55-0.99*) | 19 (33) | 0.40 (*0.20-0.79*) | 125 (53) | 0.84 (0.58-1.22) |
| Total | 78 (21) | 24 | 0.71 (*0.51-0.99*) | 7 (12) | 0.59 (*0.24-1.43*) | 71 (31) | 0.75 (0.50-1.13) |
| No surgery | 67 (19) | 2 | 3.80 (*2.66-5.43*) | - | - | - | - |
| Post-surgical treatment | | | | | | | |
| No surgery | 67 (19) | 2 | 1.00 | - | - | - | - |
| Surgery | 49 (13) | 2 | 1.25 (0.86-1.82) | 8 (14) | 1.00 | 40 (17) | 1.00 |
| Palliative | 81 (23) | 9 | 0.24 (*0.17-0.34*) | 14 (24) | 0.16 (*0.06-0.40*) | 67 (29) | 0.19 (*0.12-0.28*) |
| Curative | 163 (45) | 33 | 0.12 (*0.09-0.17*) | 36 (62) | 0.10 (*0.04-0.25*) | 127 (54) | 0.08 (*0.05-0.12*) |
| IDH1** | | | | | | | |
| Wildtype | 260 (72) | 18 | 1.00 | 36 (62) | 1.00 | 224 (96) | 1.00 |
| Mutated | 17 (5) | 59 | 0.38 (*0.21-0.68*) | 12 (17) | 0.25 (0.11-0.59) | 5 (2) | 0.92 (0.38-2.23) |

Patient characteristics, 2-year overall survival and univariate analyses for all patients, WHO grade III and WHO grade IV are shown separately. For significant variables the 95% CI are shown in italic. Abbreviations: AA: anaplastic astrocytoma, AOA: anaplastic oligo-astrocytoma, AO: anaplastic oligodendroglioma, GBM: glioblastoma multiforme. 2-y: Percentage of the patients who are alive 2 years after diagnosis. CI: confidence interval. Partial: partial resection. Total: total resection. Other: Non-frontal location. *Data missing for 9 patients (3%). **IDH1 status is missing for 67 patients (19%) with no histology and for 15 patients (4%) with inferior tissue.

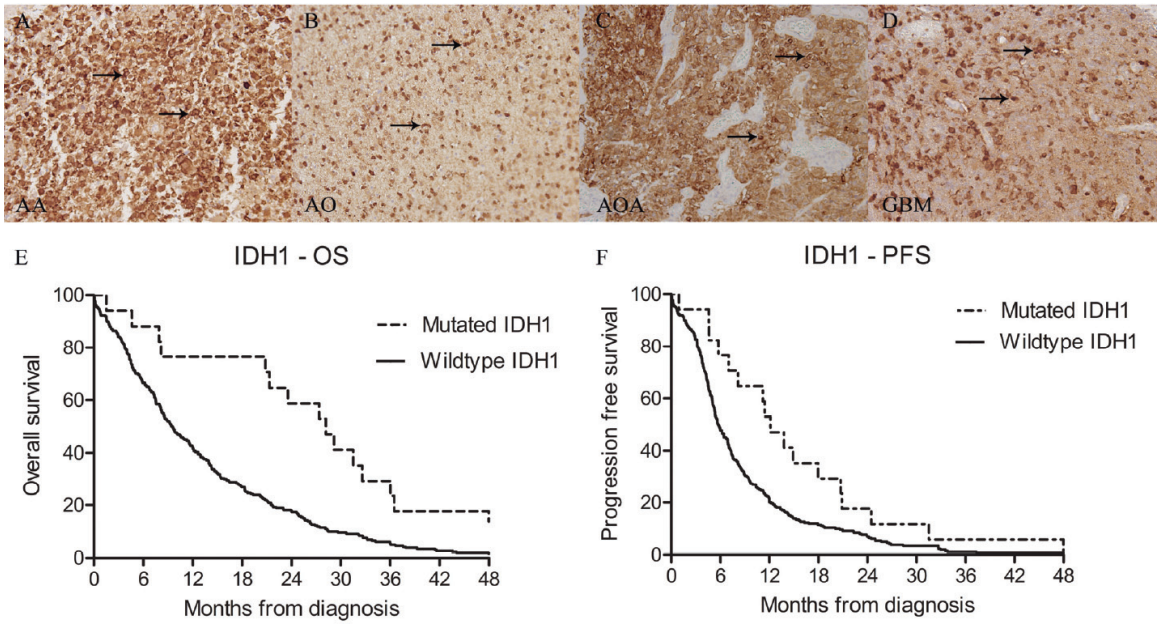


**Figure 1.** Examples of positive IDH1 staining are shown for AA (A), AO (B), AOA (C) and for GBM (D). Arrows indicate cells with mutated IDH1. Overall survival (E) and progression free survival (F) based on IDH1 status are shown. Abbreviation: AA anaplastic astrocytoma, AO anaplastic oligodendroglioma, anaplastic oligoastrocytoma, GBM Glioblastoma Multiforme.

free survival was defined as time from primary surgery until date of first recurrence, until death or until date of censoring.

*Pathology*

All tissue samples were evaluated by two independent pathologists and classified according to the World Health Organization guidelines 2007 [9]. Fresh tissue biopsies from all patients were fixed in 4% neutral buffered formalin and paraffin embedded. Three micrometer sections were cut on a microtome and stained with haematoxylin and eosin to define representative tumor regions. All sections were stained with IDH1 using the BenchMark Ultra IHC/ISH staining system (Ventana Medical Systems, Inc, AZ, USA) as follows. Sections were dried at 75°C for 4 minutes and de-paraffinized at 72°C. The staining procedure included pre-treatment with cell conditioner 1 at 99°C for 64 minutes. Slides were incubated with antibody (mIDH1R132H, clone H14, Dionova, 1: 100) at 36°C for 32 minutes. For chromogen detection, the ultraViewTM Universal DAB Detection Kit (Ventana Medical Systems) was used. Slides were removed from the BenchMark Ultra and washed in water containing soap. Slides were dehydrated and mounted.

*Statistics*

Data were described using frequencies tables; correlations were investigated with $\chi^2$-test and Spearman's correlation coefficients. The univariate relationship between prognostic and predictive variables and recurrence and death were illustrated by Kaplan-Meier plots for survival probabilities. Differences between survival functions were compared by the log-rank test. Variables significant in the univariate analysis were further analyzed in the multivariate Cox proportional hazards model. All Cox models were tested for proportional hazards, interaction-effects and time-dependency of explanatory factors. All analyses were carried out using STATA version 11.

## Results

*Patient characteristics*

The identified population comprised 359 patients of whom 292 had histologically confirmed HGG (58 with WHO grade III and 234 with WHO grade IV) and 67 were diagnosed clinically. Based on the radiology reports, all patients diagnosed clinically were considered to have a GBM. The main reasons for not offer-

**Table 2.** Multivariate analysis for all patients and for patients with tissue useable for investigation of IDH1 status

| | All | Tissue available |
|---|---|---|
| Variable | HR (95% CI) | HR (95% CI) |
| Age | 1.06 (*1.03-1.08*) | 1.04 (*1.02-1.073*) |
| Neurological deficit | | |
| Absent | 1.00 | 1.00 |
| Present | 1.35 (*1.07-1.70*) | 1.36 (*1.04-1.77*) |
| Performance status | | |
| 0-1 | 1.00 | 1.00 |
| 2-4 | 3.12 (*1.96-4.98*) | 2.39 (*1.37-4.15*) |
| Tumour crossing midline | | |
| No | 1.00 | 1.00 |
| Yes | 1.44 (*1.07-1.93*) | 1.55 (*1.09-2.21*) |
| Post-surgical treatment | | |
| No surgery | 1.00 | 1.00 |
| Surgery only | 0.72 (0.46-1.14) | 0.28 (*0.18-0.43*) |
| Palliative | 0.17 (*0.11-0.27*) | 0.16 (*0.10-0.25*) |
| Curative | 0.11 (*0.07-0.18*) | |
| IDH1 status | | |
| Wild-type | | 1.00 |
| Mutated | | 0.58 (0.32-1.07) |

ing surgery were tumor location or PS 3–4. Twelve patients declined surgery.

Median age at the time of diagnosis was 66.2 (25.8–98.0) years. At the time the data were evaluated, 26 patients (7%) were still alive. Median follow-up was 7.7 (0.01–77.7) months. Patient characteristics are described in **Table 1**.

*Progression and progression-free survival*

Three-hundred and fifty-one patients experienced a relapse, in 115 patients the first relapse resulted in death. At first and second relapse patients generally were in PS 0-1 and were capable of receiving further treatment. At relapse number three patients were in PS 2-4, and only a minority of patients received treatment. In multivariate analysis age, neurological deficits, PS, and postsurgical treatment were independent prognostic factors with regard to time to progression (data not shown).

*Overall survival*

In the entire population, 2-year OS was 18%. For patients with WHO grade III, WHO grade IV, and clinically diagnosed tumors, 2-year OSs were 29%, 19% and 2% respectively. In univariate analysis, histology, age, seizures, neurologic deficits, headache, mental disturbance, performance status, tumor crossing midline, location, extent of surgery, and postsurgical treatment were significant prognostic factors (**Table 1**).

*Isocitrate DeHydrogenase 1 (IDH1) status*

IDH1 status was investigated in 277 patients; IDH1 was mutated (mIDH1) in 17 patients (5%). mIDH1 was identified in 5/34 (15%) of patients with anaplastic astrocytoma (AA), in 4/8 (50%) of patients with anaplastic oligodendroglioma (AO), in 3/5 (60%) patients with anaplastic oligo-astrocytoma (AOA), and in 5/224 (2%) of patients with GBM (**Figure 1**).

Tissue from one patient with gliomatosis cerebri was mIDH1 negative. Patients with mIDH1 had a significantly better outcome than patients with wildtype IDH1: 2-year OS was 59% and 18%, respectively (p=0.011). In addition, there was a trend towards longer progression free survival for patients with mIDH1 than patients with wIDH1 (p=0.073) (**Figure 1**).

*Multivariate analysis*

In the best fitting multivariate Cox model young age, a tumor not crossing the midline, absence of neurological deficits, PS 0–1, and receipt of curative intended treatment were associated with better survival (**Table 2**, **Figure 2**). An interaction between age and PS was identified. There was a trend towards IDH1 status being a prognostic factor, all though it was not significant (HR 0.58, 95% CI 0.32-1.07).

*WHO grade III*

Fifty-eight patients had WHO grade III tumor. Median age at time of diagnosis was 60.4 (25.8–80.6) years. Four different sub-types of WHO grade III tumors were identified; AA, AO, AOA, and gliomatosis. Two-year OS differed significantly between the different sub-types (p=0.04).

In the majority of patients (60%), tumor was localized in a non-frontal area. These patients had a poorer outcome than patients with a frontal tumor (HR 1.96, 95% CI 1.07–3.59, p=0.030). In addition, age < 60, seizures, extent of resection, PS 0–1, and postsurgical treatment were independent prognostic factors

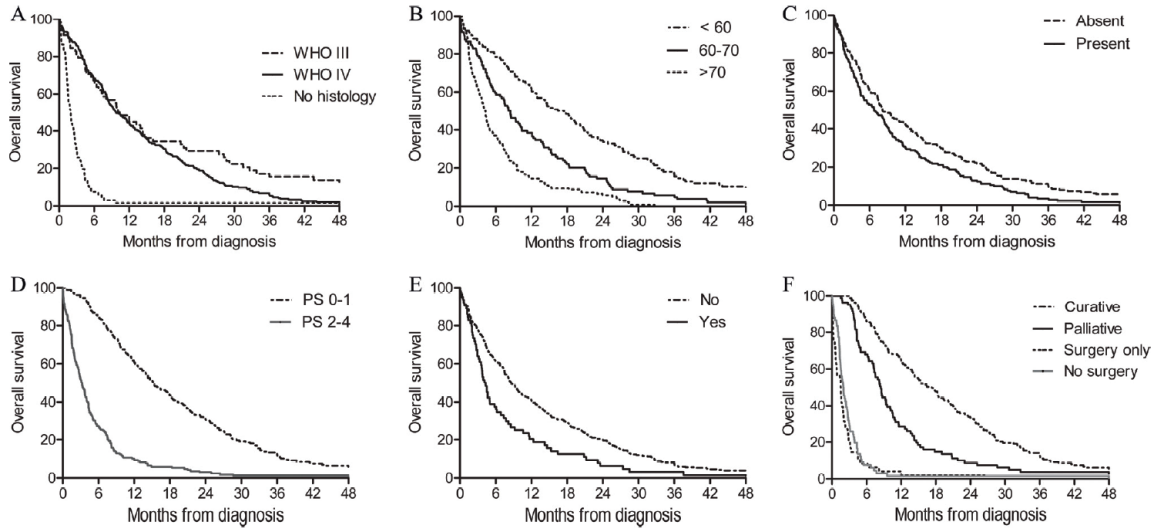


**Figure 2.** Survival curves based on histology (A), age (B), neurological deficit (C), performance status (D), tumour crossing midline (F), and treatment (G) (n=359).

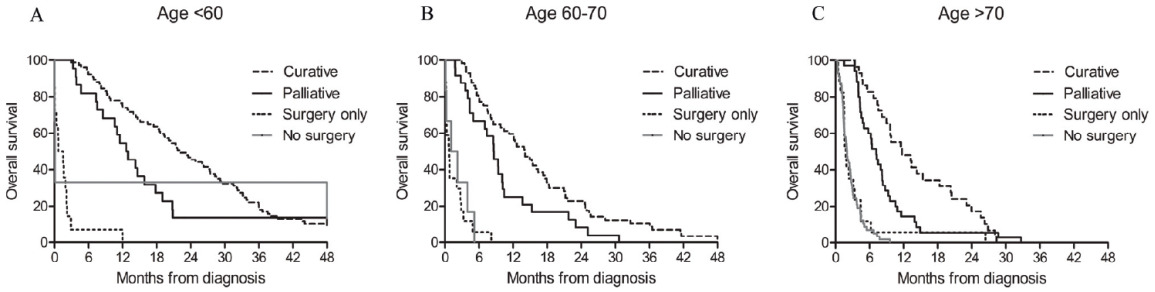


**Figure 3.** Survival curves based on treatment within three different age-groups: Patients younger than 60 years of age (A), patients 60 to 70 years of age (B), and patients older than 70 years of age (C). For each age-group treatments are defined as curative intended, palliative, surgery only and no treatment.

in patients with WHO grade III tumors in univariate analysis (**Table 1**).

*WHO grade IV*

Median age at time of diagnosis for all patients with grade IV tumors was 67.7 (33.9–98.0) years. Patients with WHO grade IV tumors were significantly younger than patients with a clinical diagnosis, with a median age at time of diagnosis of 64.7 years as compared to 81.8 years ($p<0.001$). OS was significantly shorter in patients with a clinical diagnosis ($p<0.001$) (**Figure 2**).

In univariate analyses (n=301), age, type of primary scan, tumor crossing midline, tumor diameter, headache, mental disturbance, PS, and postsurgical treatment were independent prognostic factors ($p<0.05$). For patients with WHO grade IV tumors (n=234), age, tumor crossing midline, mental disturbance, PS, and postsurgical treatment were independent prognostic factors in univariate analysis (**Table 1**).

The 2-year OS was 34% in the 102 patients with a PS 0–2, aged 18–70 years, and a histologically verified GBM given curative intended treatment. This group of patients resembled the patients in the Stupp study [3]. In our study 25 patients with a GBM received the Stupp regime, although they were > 70 years or had a PS 3–4; the 2-year OS was 20% in these patients.

The majority of patients < 60 years of age (69%) received curative intended treatment. In these patients, the 2-year OS was 42.9% compared to

6.3% in those receiving palliative treatment only (p=0.02). The majority of these patients (89%) had a PS 0–1, and in these patients the 2-year OS was 44%.

*Patients older than 70 years and time trends*

The number of patients with a high-grade glioma increased each year from 57 patients in 2005 to 92 patients in 2009, this increase was mainly seen in patients > 70 years. At the same time, a significant increase in patients receiving curative intended treatment was observed within all age groups, from 13 patients (23%) in 2005 to 50 patients (54%) in 2009 (p<0.001). A total of 163 patients received curative intended treatment during the 5-year period. The largest increase was observed in patients < 60: 8 patients (33%) received curative intended treatment in 2005 and 17 patients (71%) in 2009 (p<0.001). In patients 60-70 years of age, only 1 patient (9%) received curative intended treatment in 2005 compared to 18 patients (56%) in 2009 (p<0.001), and in patients > 70, the percentage of patients treated with a curative intent increased from 3 patients (14%) in 2005 to 10 patients (28%) in 2009 (p=0.03). In the curatively treated patients, 2-year OS was greater in patients < 60 years compared to patients 60–70 years and patients > 70 years; 2-year OSs were 47%, 23%, and 17%, respectively (p<0.001). Survival curves are shown in **Figure 3**.

The percentage of patients receiving palliative treatment decreased from 27 patients (50%) to 16 patients (17%) during the 5-year period (p<0.001). The decrease was observed mainly in patients > 70. Two-year OS increased from 0% to 14% during the period (p<0.001) in patients > 70 given palliative treatment. Palliatively treated patients had a 2-year OS that was inferior to patients given curative intended treatment (9% and 33%, respectively, p<0.001), but greater than in patients not receiving surgery or given no treatment (2% and 1.5%, respectively, p<0.001). This trend applies to all age-groups (**Figure 3**).

The percentage of patients receiving surgery or no treatment did not increase from 2005 to 2009. A total of 115 patients did not receive postsurgical treatment; these patients were characterized as having mental disturbances (51%), neurological deficits (49%), and PS 2-4 (92%). Sixty-seven of these patients (58%) were clinically diagnosed, and these patients had a particularly dismal prognosis; only 5 patients (7%) lived longer than 6 months after the primary diagnosis. Only 10 patients (15%) were younger than 70 years, and 2 patients were < 60 years. Median OS was 1.4 months in the entire group not receiving postsurgical treatment; however; one patient is still alive 80 months after the primary diagnosis.

## Discussion

We present data from a population-based retrospective study of patients with high-grade gliomas. A sub-group of patients characterized by age > 70 years, PS 2-4, and a dismal prognosis was identified. Patients with these characteristics are traditionally not included in clinical trials and information on these patients is therefore limited. We show that age, a tumor not crossing the midline, absence of neurological deficits, PS 0–1, and receiving curative intended treatment are associated with prolonged survival. In addition, we report that the prognostic effect of PS increases with increasing age.

The strengths of this study include identification of non-selected patients who were systematically reported to two Danish registries: the Danish Cancer Registry and The Danish Pathology Database. By including both histologically and non-histologically diagnosed patients, this study population better reflects patients seen in daily clinical practice, and our results can therefore be used to identify patients who will benefit from treatment.

Another strength of the study is the inclusion of 58 patients (17%) with WHO grade III tumors, a large percentage compared to other population-based studies [2]. Inclusion of WHO grade III tumors allows us to identify prognostic factors that can be used in all patients with HGGs and not only for patients with GBMs.

Furthermore, the inclusion of patients > 70 years of age allowed us to identify parameters of a subgroup of older patients who will benefit from postsurgical treatment. These patients were characterized by a PS of 0–1, a frontal tumor that did not cross the midline, as well as a higher incidence of seizures and a lower incidence of mental disturbance as their primary

symptom compared to the remaining group of patients > 70 years. Others have found that PS 0–2, absence of neurological deficits including mental deterioration, and a frontal tumor localization are associated with a favorable prognosis in glioma patients [10-12]; however, these studies did not include older patients. Kurimoto et al. [7] showed that PS is the most important prognostic factor in patients > 70 years, and Brandes et al. [5] showed that patients > 70 years with good PS do benefit from treatment, which is in accordance with our results.

However, we also show that patients < 60 benefitted more from postsurgical treatment than older patients. This is in accordance with a recent population-based study by Lawrence et al. [1]. They identified 13,003 patients with GBM and reported that OS decreased with increasing age. In addition they showed that OS increased from 2001 to 2007, but only in patients < 70 years. The fact that patients > 70 respond less well to treatment than younger patients has been described before [2, 13, 14], and several approaches have been attempted to improve survival in glioma patients > 70 years [5, 6, 8].

In our study, 102 GBM patients resembled the patients included in the EORTC study [3], and we found a 2-year OS of 34 %, which is higher than the 27% reported in the experimental arm of the EORTC study [3] and the 21% reported by Lawrence et al. [1]. The difference between our study and that of Lawrence et al. may be the use of the SEER database, which does not contain information on PS or concomitant temozolomide, and the authors suggest that 2-year OS might increase if these variables were taken into account. Furthermore, there may be differences in the treatment given on progression of symptoms. This also applies to the patients in the EORTC study.

The extent of resection is another accepted prognostic factor [10, 11, 15]. In our study this was not a prognostic factor, probably because the parameter was based on the surgeons' opinion and not on a postsurgical scan. This is in accordance with a study by Iliadis et al. [16] that included only patients receiving radiotherapy and temozolomide. They concluded that only the postsurgical enhancing tumor volume and PS had an impact on OS, whereas the impact of PS on survival was less evident than seen in our study of non-selected patients.

Patients with mIDH1 have a better prognosis than patients with wild-type IDH1 in the univariate analysis, but the prognostic effect of mIDH1 was not significant in the multivariate analysis. In our study, only 2% of GBM patients carried the mutation, which is below the percentage reported in some studies [17, 18] but comparable to others [19, 20]. Also, the percentage of patients with WHO grade III tumors and mIDH1 was smaller in our study than in the study presented by Balss et al. [17]. The difference may be due to different methodologies because both Balss et al. [17] and Bleeker et al. [18] used sequencing, whereas Mellai et al. [20] and Combs et al. [19] used immunohistochemistry. In 2011, Takano et al, [21] compared the use of sequencing with IHC to detect mIDH1. A correlation of 83% was reported, a result supported by Capper et al. [22]. When IHC is used, only the most frequent mutation (R132H) is identified. Direct sequencing identifies other mutations (R123C, R132G, R132S, and R132L) as well, and this may explain the higher percentage of mIDH1 found with direct sequencing than with IHC and thereby the lower frequency of mIDH1 found in our study.

In conclusion, we describe the total population of patients with high-grade gliomas, including a subpopulation of patients, who never received any treatment. We identified age, PS 0–1, absence of neurological deficit, having a tumor that does not cross the midline, and receiving postsurgical treatment as independent indicators of a good prognosis. In addition, patients carrying a mutated IDH1 have a better outcome as compared to patients with wild-type IDH1 in univariate analysis; although the prognostic effect was not statistically significant when adjusted for other factors.

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## References

[1] Lawrence YR, Mishra MV, Werner-Wasik M, Andrews DW, Showalter TN, Glass J, Shen X, Symon Z and Dicker AP. Improving prognosis of glioblastoma in the 21st century: Who has benefited most? Cancer 2011.

[2] Ohgaki H and Kleihues P. Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. J Neuropathol Exp Neurol 2005; 64: 479-489.

[3] Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E and Mirimanoff RO. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005; 352: 987-996.

[4] Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, Ludwin SK, Allgeier A, Fisher B, Belanger K, Hau P, Brandes AA, Gijtenbeek J, Marosi C, Vecht CJ, Mokhtari K, Wesseling P, Villa S, Eisenhauer E, Gorlia T, Weller M, Lacombe D, Cairncross JG and Mirimanoff RO. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 2009; 10: 459-466.

[5] Brandes AA and Monfardini S. The treatment of elderly patients with high-grade gliomas. Semin Oncol 2003; 30: 58-62.

[6] Keime-Guibert F, Chinot O, Taillandier L, Cartalat-Carel S, Frenay M, Kantor G, Guillamo JS, Jadaud E, Colin P, Bondiau PY, Menei P, Loiseau H, Bernier V, Honnorat J, Barrie M, Mokhtari K, Mazeron JJ, Bissery A and Delattre JY. Radiotherapy for glioblastoma in the elderly. N Engl J Med 2007; 356: 1527-1535.

[7] Kurimoto M, Nagai S, Kamiyama H, Tsuboi Y, Kurosaki K, Hayashi N, Origasa H and Endo S. Prognostic factors in elderly patients with supratentorial malignant gliomas. Neurol Med Chir (Tokyo) 2007; 47: 543-549.

[8] Lutterbach J and Ostertag C. What is the appropriate radiotherapy protocol for older patients with newly diagnosed glioblastoma? J Clin Oncol 2005; 23: 2869-2870.

[9] WHO Classification of Tumours of the Central Nervous System. Edited by Louis DN, Ohgaki H, Wiestler OD and Cavenee WK. International Agency for Research on cancer (IARC) 2007.

[10] Casartelli G, Dorcaratto A, Ravetti JL, Sola S, Vitali A, Merlo DF and Frosina G. Survival of high grade glioma patients depends on their age at diagnosis. Cancer Biol Ther 2009; 8: 1719-1721.

[11] Martinez R, Volter C and Behr R. Parameters assessing neurological status in malignant glioma patients: prognostic value for survival and relapse-free time. Br J Neurosurg 2008; 22: 557-562.

[12] Watne K, Hannisdal E, Nome O, Hager B and Hirschberg H. Prognostic factors in malignant gliomas with special reference to intra-arterial chemotherapy. Acta Oncol 1993; 32: 307-310.

[13] Lacroix M, Abi-Said D, Fourney DR, Gokaslan ZL, Shi W, DeMonte F, Lang FF, McCutcheon IE, Hassenbusch SJ, Holland E, Hess K, Michael C, Miller D and Sawaya R. A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. J Neurosurg 2001; 95: 190-198.

[14] Shirai K and Chakravarti A. Towards personalized therapy for patients with glioblastoma. Expert Rev Anticancer Ther 2011; 11: 1935-1944.

[15] Kim KJ, Lee KH, Kim HS, Moon KS, Jung TY, Jung S and Lee MC. The presence of stem cell marker-expressing cells is not prognostically significant in glioblastomas. Neuropathology 2011.

[16] Iliadis G, Kotoula V, Chatzisotiriou A, Televantou D, Eleftheraki AG, Lambaki S, Misailidou D, Selviaridis P and Fountzilas G. Volumetric and MGMT parameters in glioblastoma patients: survival analysis. BMC Cancer 2012; 12: 3.

[17] Balss J, Meyer J, Mueller W, Korshunov A, Hartmann C and von DA. Analysis of the IDH1 codon 132 mutation in brain tumors. Acta Neuropathol 2008; 116: 597-602.

[18] Bleeker FE, Lamba S, Leenstra S, Troost D, Hulsebos T, Vandertop WP, Frattini M, Molinari F, Knowles M, Cerrato A, Rodolfo M, Scarpa A, Felicioni L, Buttitta F, Malatesta S, Marchetti A and Bardelli A. IDH1 mutations at residue p.

R132 (IDH1(R132)) occur frequently in high-grade gliomas but not in other solid tumors. Hum Mutat 2009; 30: 7-11.

[19] Combs SE, Rieken S, Wick W, Abdollahi A, von DA, Debus J and Hartmann C. Prognostic significance of IDH-1 and MGMT in patients with glioblastoma: one step forward, and one step back? Radiat Oncol 2011; 6: 115.

[20] Mellai M, Piazzi A, Caldera V, Monzeglio O, Cassoni P, Valente G and Schiffer D. IDH1 and IDH2 mutations, immunohistochemistry and associations in a series of brain tumors. J Neurooncol 2011; 105: 345-357.

[21] Takano S, Tian W, Matsuda M, Yamamoto T, Ishikawa E, Kaneko MK, Yamazaki K, Kato Y and Matsumura A. Detection of IDH1 mutation in human gliomas: comparison of immunohistochemistry and sequencing. Brain Tumor Pathol 2011; 28: 115-123.

[22] Capper D, Weissert S, Balss J, Habel A, Meyer J, Jager D, Ackermann U, Tessmer C, Korshunov A, Zentgraf H, Hartmann C and von DA. Characterization of R132H mutation-specific IDH1 antibody binding in brain tumors. Brain Pathol 2010; 20: 245-254.