

PROGNOSTIC IMPLICATION OF CLINICAL AND PATHOLOGIC FEATURES IN PATIENTS WITH GLIOBLASTOMA MULTIFORME TREATED WITH CONCOMITANT RADIATION PLUS TEMOZOLOMIDE

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Aims and background: Glioblastoma multiforme is the most common and most malignant primary brain tumor in adults. The current standard of care for glioblastoma is surgical resection to the extent feasible, followed by adjuvant radiotherapy plus temozolomide, given concomitantly with and after radiotherapy. This report is a prospective observational study of 43 cases treated in the Department of Radiotherapy, University of Rome La Sapienza, Italy. We examine the relationship between pathologic features and objective response rate in adult patients treated with concomitant radiation plus temozolomide to identify clinical, neuroradiologic, pathologic, and molecular factors with prognostic significance.

Methods: Forty-three consecutive patients (24 males and 19 females), ages 15-77 years (median, 57) with newly diagnosed glioblastoma multiforme, were included in this trial between 2002 and 2004 at our department. All patients were treated with surgery (complete resection in 81%, incomplete in 19%) followed by concurrent temozolomide (75 mg/m²/day) and radiotherapy (median tumor dose, 60 Gy), followed by temozolomide, 200 mg/m²/day for 5 consecutive days every 28 days. Neurologic evaluations were performed monthly and cranial magnetic resonance bimonthly. We analyzed age, clinical manifestations at diagnosis, seizures, Karnofsky performance

score, tumor location, extent of resection, proliferation index (Ki-67 expression), p53, platelet-derived growth factor and epidermal growth factor receptor immunohistochemical expression as prognostic factors in the patients. The Kaplan-Meier statistical method and logrank test were used to assess correlation with survival.

Results: Fourteen patients (32%) manifested clinical and neuro-radiographic evidence of tumor progression within 6 months of surgery. In contrast, 5 patients (12%) showed no disease progression for 18 months from the beginning of treatment. Median overall survival was 19 months. Multivariate analysis revealed that an age of 60 years or older ($P < 0.03$), a postoperative performance score ≤ 70 ($P = 0.04$), the nontotal tumor resection ($P = 0.03$), tumor size > 4 cm ($P = 0.01$) and proliferation index overexpression ($P = 0.001$) were associated with the worst prognosis. p53, PDGF and EGFR overexpression were not significant prognostic factors associated with survival.

Conclusions: The results suggest that analysis of prognostic markers in glioblastoma multiforme is complex. In addition to previously recognized prognostic variables such as age and Karnofsky performance score, tumor size, total resection and proliferation index overexpression were identified as predictors of survival in a series of patients with glioblastoma multiforme.

Key words: EGFR, glioblastoma multiforme, Ki67, p53, PDGF, prognostic factor, temozolomide.

Introduction

Astrocytic tumors are the most common glial neoplasms, with an annual incidence of 3-4/100,000 inhabitants, and approximately 80% are glioblastomas¹. High-grade gliomas are among the most devastating types of astrocytic tumors. Glioblastoma patients have a median survival of 12 months following surgical resection and radiotherapy. In a meta-analysis, chemotherapy resulted in an approximate increase of 2 months in the median survival time²⁻⁴. Of the chemotherapeutic drugs used to treat glioblastoma, alkylating agents are the most widely used. In one study, temozolomide, compared with procarbazine, not only prolonged progression-free survival (PFS), but also maintained neurological functioning and performance status for a longer period of time⁵.

Temozolomide is an imidazotetrazine derivative of the alkylating agent decarbazine with virtually complete oral bioavailability and enhanced ability to cross the blood-brain barrier. Given concurrently and following radiotherapy, it increased median survival from 14 to 18

months in a recent randomized trial⁶. Temozolomide is generally well tolerated and, unlike other alkylating agents, easily administered orally. The activity of temozolomide is highly dependent on dosing schedule, with multiple administrations being more effective than a single dose. In fact, laboratory data showed that temozolomide cytotoxicity was more pronounced using protracted exposures. With this regimen, the drug penetrates the blood-brain barrier to reach the central nervous system in quantitatively sufficient doses. The response appears to be related to tumor histology. Alkylators like temozolomide exert their cytotoxic effect through methylation of DNA bases. DNA damage is repaired by direct reversal of the O6-MeG lesion by the protein O6-methylguanine-DNA methyltransferase. Recognition of alkylation-damage-induced has been associated with replication errors by the mismatch repair pathway and excision repair of alkylated bases (N7-MeG and N3-MeA) conducted by the Base Excision Repair (BER) pathway. The presence of a functional mis-

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Received September 7, 2006; accepted January 16, 2007.

match repair pathway is a requirement for the cytotoxic effect of alkylating agents and is significantly correlated with clinical response. Decreased base excision repair protein expression is correlated with increased sensitivity to alkylator-based chemotherapy.

Glioblastoma multiforme (GBM) is regarded as phenotypic and an indication of diffusely infiltrating astrocytoma. According to the World Health Organization (WHO) classification of neural tumors, the typical histologic appearance of the tumor requires hypercellularity, nuclear polymorphism, brisk mitotic activity, prominent microvascular proliferation, and/or necrosis, and it corresponds to WHO grade IV⁷.

Glioblastomas arise either *de novo* (primary glioblastomas) or from pre-existing lower grade astrocytic tumors (secondary glioblastomas). Primary and secondary glioblastomas are considered to be histologically indistinguishable, but the patients' mean age at diagnosis is approximately 55 years for primary and 40 years for secondary glioblastoma⁸.

Primary glioblastomas have a high rate of overexpression or mutation of the gene encoding the epidermal growth factor receptor (EGFR), mutation of the gene for phosphatase and tensin homologues, alterations of the cellular oncogene murine double minute, and tumor suppressor gene p16 deletions. In contrast, secondary glioblastomas often have alterations of TP53 and overexpression of platelet-derived growth factor- α (PDGF α) and its receptor (PDGFR α)⁷⁻¹².

The MIB-1 antibody detects the Ki-67 antigen, which is expressed only in actively cycling cells¹³. Although Ki-67 labeling index predicts prognosis in low-grade astrocytomas^{14,15}, studies in patients with GBM are less clear¹⁶⁻¹⁸.

Even though many predictive prognostic factors have been considered, at present only a young age and good neurologic performance are commonly regarded to promise longer survival. More recent studies have demonstrated the importance of the extent of resection and Karnofsky performance status (KPS) as prognostic factors at the time of diagnosis as well as after treatment with chemotherapy or radiotherapy¹⁹⁻²².

The aim of the present study was to present and discuss clinical features and prognostic factors in a series of patients with GBM treated with radiation therapy and concomitant temozolomide and to compare the results with those of the literature.

Patients and methods

We investigated the clinical characteristics, functional outcomes, and survival in 43 consecutive adult patients with GBM (WHO Grade IV). They were diagnosed and treated between January 2002 and December 2004 at the University of Rome "La Sapienza", in the Department of Radiotherapy.

The median age was 57 years (range, 15-77); 24 patients were male and 19 female. The characteristics of the 43 patients are listed in Tables 1-3.

Table 1 - Characteristics of 43 patients with glioblastoma

	Variable	No. of patients
Age (yr)	≥60	25
	<60	18
Sex	Male	24
	Female	19
Barthel index	10-70	3
	75-100	40
Resection status	Total resection	35
	Non-total resection	8
Postoperative MR	Negative	16
	Positive	27

MR, magnetic resonance.

Table 2 - Characteristics of tumors

	No. of patients
Location of tumor	
Frontal lobe	11
Temporal lobe	17
Parietal lobe	7
Occipital lobe	3
Brainstem	2
Corpus callosum	3
Hemisphere	
Left	23
Right	17
Median line	3
Tumor size (cm)	
>4	18
≤4	25
Ki67 PI	
>15%	20
≤15%	19
P53	
Pos	20
Neg	19
PDGF	
Pos	22
Neg	17
EGFR	
Pos	16
Neg	23

Table 3 - Karnofsky performance status (KPS)

	No. of patients
Pre-RT performance status (KPS)	
100-70	34
<70	9
Post-RT performance (KPS)	
100-70	29
<70	14

All patients were managed according to a previously established common diagnostic and therapeutic protocol. The protocol included adjuvant radiotherapy and chemotherapy after surgical resection. All patients underwent neurosurgical treatment prior to radiochemotherapy,

and all patients were operated at our institution. In 35 patients, a clinically complete resection was performed, and in 16 of these postoperative magnetic resonance imaging (MRI) was negative for the presence of disease. In 19 patients, residual tumor was still present in postoperative MRI after radical neurosurgery. In one of 35 patients, the tumor was localized in the brainstem; a surgical approach in this site is uncommon, but, in this case, a radical surgery was possible for a tumor <8 mm in diameter and its uncritical location. Subtotal resection of the tumor was performed in 8 patients (Table 1).

Focal radiotherapy was delivered once daily at 2 Gy per fraction, 5 days/week, for a total of 60 Gy, and was prescribed according to the guidelines of the International Commission on Radiological Units. Adequate immobilization masks were required to ensure reproducibility. Treatment volumes were determined on the basis of preoperative contrast-enhanced computerized tomography (CT) or gadolinium-enhanced MRI of the brain. The initial step in the treatment planning process is the identification of the gross tumor volume, which is the enhancing gross tumor seen on preoperative MRI or CT with contrast. The clinical target volume represents the volume of tissue at risk for harboring microscopic tumor and typically contains the edematous brain tissue surrounding a contrast-enhancing lesion plus a 1 to 2-cm margin, depending on the location. The planning target volume, which usually extends 0.5 to 1 cm beyond the margin of the gross tumor volume and clinical target volume, accounts for factors such as internal organ motion, setup variation, and patient movement. The treated volume was encompassed by the 95% isodose volume. Planning for radiotherapy always included dedicated CT, three-dimensional reconstruction with treatment planning computation (Pinnacle System), and beam's-eye-view for the choice of treatment field number, size, and shape. Radiation therapy was delivered with 6 MV photons from a linear accelerator.

Concomitant treatment with temozolomide was prescribed at a dose of 75 mg/m² on each day of radiation treatment for 13 patients and 7 days per week for 30 patients. It was administered orally, in a fasting state, once a day for 5 or 7 consecutive days per week, from the first to the last day of radiotherapy. Application was early in the morning at approximately the same time of the day within and during each week.

Four weeks after radiotherapy, patients received adjuvant temozolomide chemotherapy, 150 to 200 mg/m² on days 1 to 5 at 28-day intervals. Treatment continued until progression of tumor or toxicity developed.

Antiemetic prophylaxis using metoclopramide or a 5-hydroxytryptamine antagonist was recommended before the initial doses of concomitant temozolomide and was also required during the adjuvant 5-day courses of temozolomide. Anticonvulsants and corticosteroids were administered as needed.

After treatment, all patients were followed in the outpatient clinic until death or last visit. Patients were examined one month after completion of chemo-radio-

therapy and every 3 months thereafter. The baseline examination included MRI, full blood counts and blood chemistry tests, a physical examination and KPS evaluation. An initial MRI scan was performed within 2 weeks prior to commencing chemotherapy. Subsequent scans were performed every 2 months in the first year and every 3 months in the second year or when a tumor recurrence was suspected.

The primary end point was estimation of PFS and overall survival (OS); a secondary end point was safety and quality of life. PFS was the interval between surgery and tumor progression, defined according to modified WHO criteria, on the basis of measurement in relation to baseline MRI as an increase in tumor size of 25%, the appearance of a new lesion, or an increased need for corticosteroids. OS was defined from the time of primary diagnosis to the date of last follow-up visit or death for tumor-related reasons.

Statistical analysis was performed using the MINITAB 14.0 software package. Quantitative variables and duration of follow-up were expressed as median and range. Univariate analysis was performed by constructing probability curves according to the Kaplan-Meier method and comparing them using the logrank test. The variables that were considered and evaluated as most likely to be related to PFS and OS were age at diagnosis (>60 vs ≤60), preradiotherapy KPS (≥70 vs <70), residual disease after surgery, tumor location (right, left or median line), proliferation index (Ki67 expression >20% vs ≤20%), p53, GFAP, EGFR immunohistochemical expression, and tumor size (<4 cm vs ≥4 cm).

Immunohistochemical studies for p53, EGFR, and Ki-67 antibodies and overexpression of PDGF α were performed in 39 of 43 patients (90%). The proliferation index was determined by immunohistochemical staining for Ki67 using the MIB-1 antibody.

Results

Forty-three patients underwent neurosurgical treatment prior to radiochemotherapy, from January 2002 until December 2004, at the University of Rome "La Sapienza", in the Department of Radiotherapy. The main clinical characteristics of the 43 patients are summarized in Table 1. Thirty-eight patients presented with neurologic symptoms before and 30 patients after radiochemotherapy, including motor and sensory deficits, headache, nausea and vomiting, epilepsy and edema with increased intracranial pressure (Table 2). Tumor location and histological features are reported in Table 3. The KPS was ≥70 in 34 patients and <70 in 9 patients before treatment began, and ≥70 in 29 and <70 in 14 patients after completion of treatment (Table 3). Surgery was performed from an average of 4 weeks from the diagnosis (range, 2-8). The median time between surgical treatment and the beginning of radiochemotherapy was 2.3 months (range, 2-4).

At the time of analysis, 16 of 43 patients (37%) were still alive, with a medium follow-up of 23 months

(range, 5-35). One patient was lost to follow-up. Fourteen patients (32%) manifested clinical and neuroradiographic evidence of tumor progression within 6 months of surgery. In contrast, 5 patients (12%) showed no disease progression for 18 months from the beginning of treatment.

The median PFS was 11 months (95% confidence interval, 9.2-13.2), with a 1-year and 2-year probability of PFS of 44% and 6%, respectively (Figure 1).

The average OS was 19 months (95% confidence interval, 16.8-22.8) with a 1-year and 2-year probability of survival of 71% and 21%, respectively (Figure 2).

All 43 patients were evaluated for radiochemotherapy toxicity according to WHO criteria. We observed grade 2 and 3 thrombocytopenia in 9 patients (20%), whereas neutropenia, leukopenia or anemia of grade 3 were reported in less than 9%. Nonhematological toxicity was mainly transaminase elevation grade I to II in 24% of patients and nausea and vomiting grade I to II in 31%. Five patients reported fits of dizziness and headache.

The relation of histological variables to survival was investigated for all 43 patients. The proliferation index measured by labeling of Ki-67 was >15% in 20 patients

(51%). Overexpression of EGFR was detected in 31% of patients, whereas p53 overexpression was detected in 25 of 38 patients (65%). Finally, PDGF overexpression was observed in 52% of patients. The parameter was significantly associated with p53 overexpression (51%), whereas no correlation was observed for the other molecular markers (EGFR and Ki-67).

The influence of predictive factors on survival was further evaluated by calculating an index of probability of survival to include the independent variables. To calculate this index, each variable was scored as 0 or 1 according to the absence or presence of the favorable category.

Kaplan-Meier curves of significant associations are shown in Figures 3 and 4. Five variables were significantly associated with prolonged survival: a) age, younger than 61 years ($P = 0.03$); b) pre-radiotherapy KPS equal to or greater than 70 ($P = 0.04$); c) total tumor resection ($P = 0.03$), d) tumor size <4 cm ($P = 0.01$); e) Ki 67 <15% ($P = 0.001$). There was no association of p53 ($P = 0.06$), PDGF ($P = 0.08$), or EGFR (0.06) with survival.

Discussion

GBM is a biologically aggressive neoplasm, with a median survival of 12-14 months following initial diagnosis. Although considerable progress has been made in the treatment of these tumors with combinations of surgery, radio- and chemotherapy, almost all patients with malignant glioma die of their tumor, and OS time is comparably low.

The value of radiotherapy was established in randomized trials in the late 1970s. Surgical resection followed by postoperative local-regional radiotherapy is considered the current standard approach to the treatment of malignant glioma²³⁻²⁵. In recent years, a remarkable re-orientation has been taking place in the treatment of malignant gliomas. Recent evaluations of radiochemotherapy regimens have proven a beneficial outcome for this multimodality approach, resulting in a prolongation of median OS, with chemotherapy being a part of the first-line treatment in patients with malignant gliomas. A combination of radiotherapy with oral and intravenous administration of procarbazine, CCNU, and vincristine (PVC) could slightly increase OS^{26,27}. The downside of PVC application is the high rate of side effects including severe neuropathy.

An RTOG phase I trial, in which 47 GBM patients were treated with concomitant radiotherapy plus topotecan, reported a median survival of 9.7 months²⁸. Similarly, 124 GBM patients treated with concomitant radiotherapy plus tirapazamine, a hypoxia-selective cytotoxin, had a median survival of approximately 10 months²⁹. Furthermore, Kleinberg *et al.*³⁰ reported a median survival of 12.8 months for patients treated with concomitant radiotherapy plus cisplatin and BCNU. Since most recurrences in malignant gliomas occur within 2 cm of the previous resection, local chemotherapy with

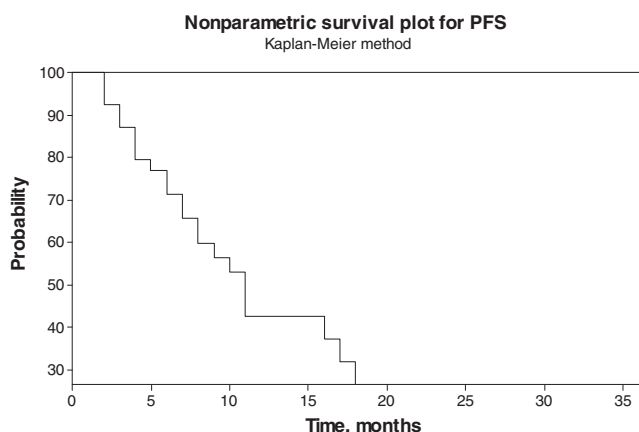


Figure 1 - Progression-free survival.

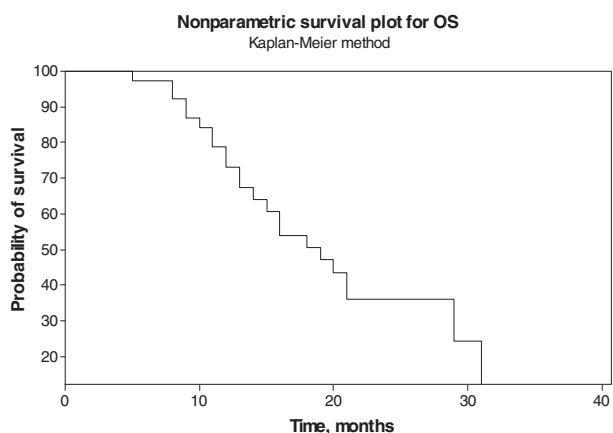


Figure 2 - Overall survival.

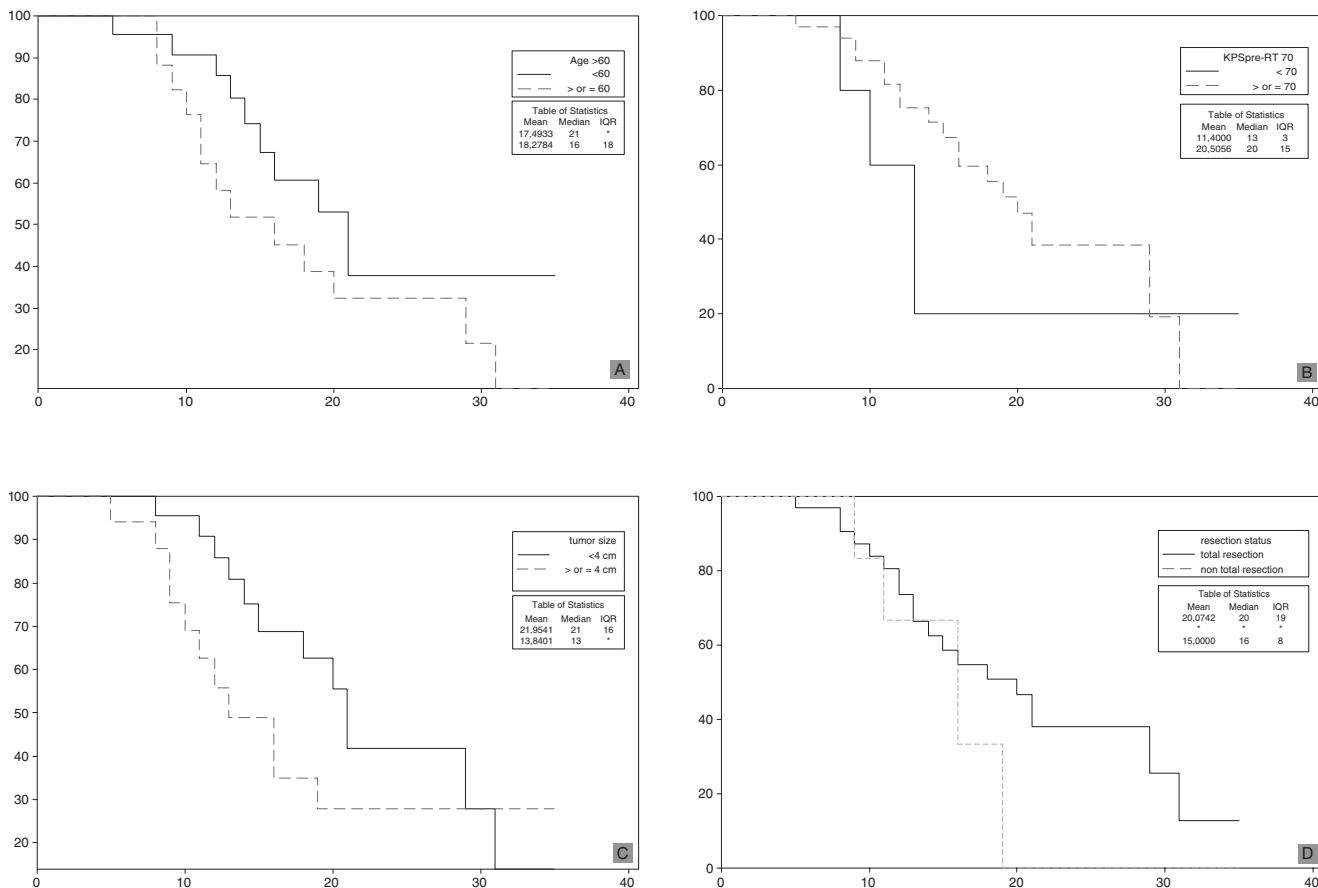


Figure 3 - Graphs A to D depict total survival times in relation to clinical parameters (univariate analysis): A) Age: <60 years vs >60 years ($P = 0.03$); B) pre-radiation therapy KPS: ≥ 70 vs <70 ($P = 0.04$); C) tumor size: ≥ 4 cm vs <4 cm ($P = 0.01$); D) resection status: total resection vs non total resection ($P = 0.03$).

biodegradable polymers has been investigated in patients with recurrent gliomas³¹. In a randomized phase III trial with newly diagnosed glioma patients, BCNU polymers were implanted during the initial resection, and then patients were subsequently treated with standard radiotherapy. Preliminary analysis indicated that patients implanted with BCNU polymers had a prolonged median survival (13.9 months; $P = 0.08$) compared with patients implanted with placebo polymers (11.6 months)³².

Application of teniposide (VM26) in addition to BCNU and concomitant irradiation has been shown to further extend PFS in a trial of the GAG Group, but AN influence on OS was not observed (GAG Study Group). However, BCNU administration involves a high risk of pulmonary toxicity, which provided the rationale to substitute nimustine (ACNU) for BCNU. The NOA-1 trial confirmed the notion that concomitant application of ACNU plus ARA-C or teniposide (VM26) is beneficial in the treatment of primary malignant glioma without causing relevant pulmonary toxicity³³.

The effect of concomitant application of temozolomide with irradiation is supported by results reported in

the literature. 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 initiated a randomized, multicenter, phase III trial, published by Stupp *et al.*⁶, to compare this regimen with radiotherapy alone in patients with newly diagnosed glioblastoma. Recently, the results of this large randomized phase III clinical trial were presented, demonstrating a significantly increased OS in patients who received radiochemotherapy with 75 mg/m² temozolomide and up to six cycles of adjuvant temozolomide compared to patients treated with irradiation alone. OS could be increased from 12.1 to 14.6 months. The median PFS was 6.9 months with radiochemotherapy and 5 months with radiotherapy alone.

In our study, we examined the safety and efficacy of concomitant radiotherapy plus temozolomide followed by adjuvant temozolomide in 43 patients with GBM, confirming the overall excellent tolerability of the regimen. In line with the literature, we obtained a median OS of 19 months, with a 1-year and 2-year probability of survival of 71% and 21%, respectively, and a medi-

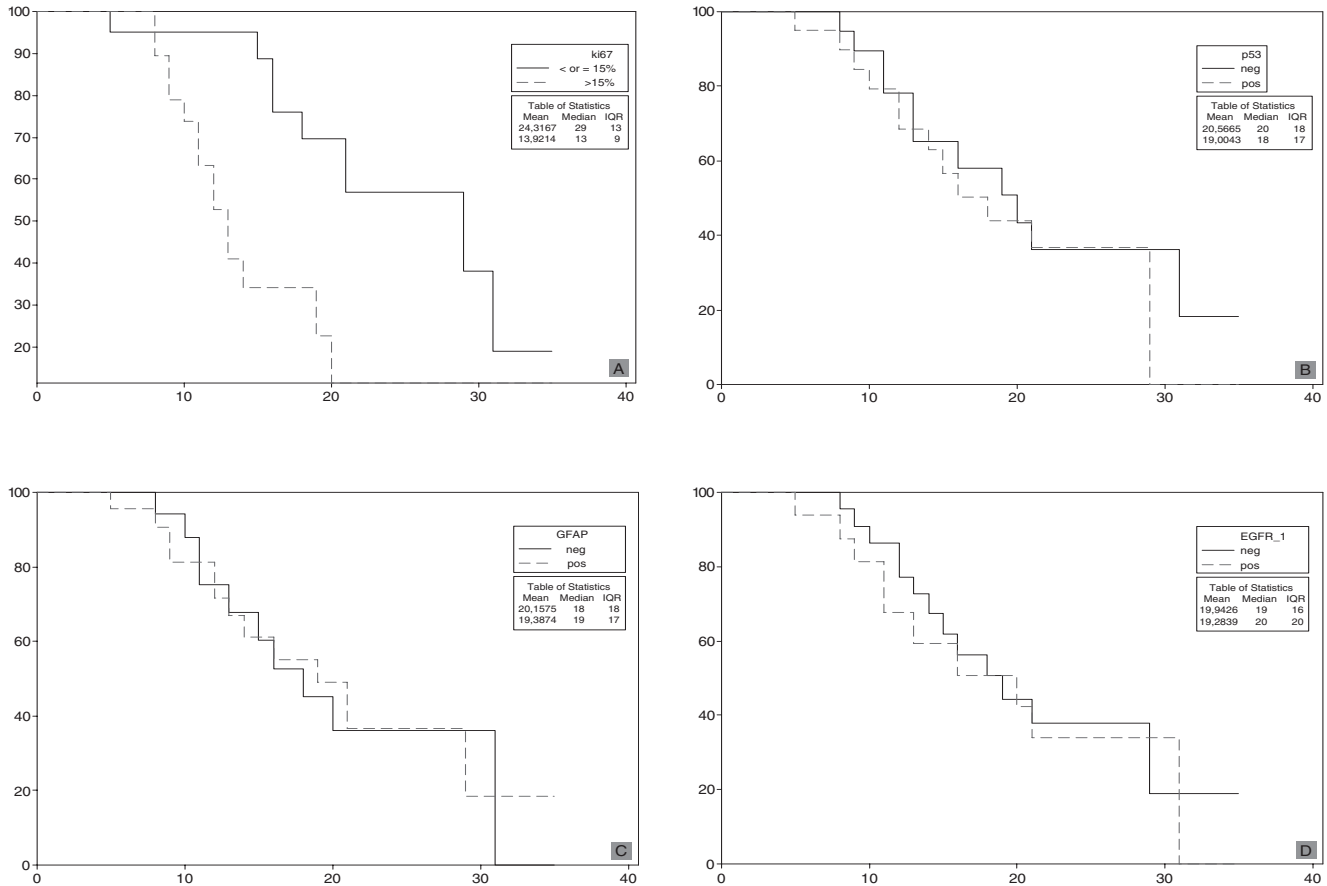


Figure 4 - Graphs A to D depict total survival times in relation to clinical parameters (univariate analysis): A) Ki67 >15% vs <15% ($P = 0.001$); B) p53 positive vs negative ($P = 0.06$); C) GFAP positive vs negative ($P = 0.08$); D) EGFR positive vs negative ($P = 0.06$).

an PFS of 11 months, with a 1-year and 2-year probability of PFS of 44% and 6%, respectively. Patients under 61 years of age had a significantly prolonged survival ($P = 0.03$). As a prognostic variable, age constitutes a continuous spectrum, with the survival rate decreasing with increasing age. Moreover, the results of the present study suggest that initial KPS is a predictive factor of survival in patients with GBM ($P = 0.04$). In addition, as previously reported, total tumor resection and tumor size may also constitute favorable prognostic factors. Finally, proliferation index, established by labeling of Ki-67, also had prognostic influence in this series.

With regard to the strong relationship between some prognostic factors and length of survival, a nonparametric recursive partitioning analysis uses age, histology, KPS, mental status, duration of symptoms, neurological functional class, extent of surgery, and radiation dose. All these significant partitioning covariates were identified in the evaluation of 1578 patients in three successive Radiation Therapy Oncology Group malignant glioma trials to define six different classes that can be lumped into three prognostic subgroups: a favorable

prognosis subgroup (12% of patients) consisting of classes I and II with median survivals of 37 to 59 months and 2-year survival rates of 68% to 76%; an intermediate prognosis subgroup (43% of patient) consisting of classes III and IV with a median survival of 11 to 18 months and 2-year survival rate of 15% to 35%; and a poor prognosis subgroup (45% of patients) consisting of classes V and VI with a median survival of 4.6 to 9 months and 2-year survival rates of 4% to 6%. Patients aged 60 years or older have the worst prognosis, with an 18-month survival rate of 8%. Patients from 40 to 59 years of age have a 18-month survival rate of 20%. Patients younger than 40 years have an 18-month survival rate of 60%. When evaluated by recursive partitioning analysis, the age of 50 years constitutes the major dividing point³⁴.

KPS has also been established as one of the most important prognostic factor for malignant gliomas. Review of the literature suggests that patients with low a KPS tend to do significantly worse than those with higher KPS³⁵. In the RTOG-ECOG trial, the 18-month survival for patients with a KPS of 70 was 34% compared with 10%-13% for those with a KPS of less than 70³⁶.

Surgical extent of resection is controversial as a prognostic factor because patients who are most amenable to complete tumor resection are either younger patients with good KPS or those with small tumors that are not adjacent to critical structures. However, several studies suggest that gross tumor resection or subtotal tumor resection is better than biopsy alone. Combined analysis of three RTOG trials, including RTOG 74-01, RTOG 79-18 and RTOG 83-02, suggests that patients undergoing biopsy alone had a significantly worse tumor outcome than those undergoing gross tumor resection (median survival, 6.6 vs 11.3 months). However, in evaluating these results, the possibility of patient selection bias must be kept in mind^{37,38}. Our results show that the mean survival in patients who underwent a total resection was 20 vs 15 months in those with nontotal resection ($P = 0.03$).

Many investigations have focused on glioma behavior. Their goals were to identify molecular factors correlating with histologic features and to predict clinical outcome. Unfortunately, the results have been quite poor. However, it is possible that these markers are important in defining genetic subgroups with regard to tumor biology and possibly treatment selection. Prognostic factors attributable to both the patient and the therapeutic strategies involved have been investigated in several trials. Previous studies have suggested that GBM can be defined as primary versus secondary based on clinical characteristics (initial histologic diagnosis and length of symptoms before initial surgery). This distinction is supported by mutually exclusive EGFR amplification and p53 mutation, respectively. In several studies, EGFR overexpression or amplification has been shown to be associated with a poor prognosis³⁹⁻⁴². Other studies found no such prognostic relationship⁴³⁻⁴⁶.

Most investigations have found that p53 mutation or overexpression is not a significant prognostic factor for survival in GBM^{40, 47-52}.

The Ki67/MIB1 cell marker recognizes a nuclear antigen specific for proliferating cells in the G1, S, G2, and M phases of the cell cycle. It is routinely used to estimate the growth fraction in gliomas. Mean values are 15% to 20%. The overexpression of Ki67 proliferation index was not associated with survival in most of the studies. Proliferation indices have not been consistently demonstrated to be associated with prognosis in high-grade astrocytomas¹⁴⁻¹⁸.

No consensus has been established on the prognostic value of PDGFN. Some groups claim no association with survival and others claim that this aberration is a negative prognostic factor²⁰. It is possible that differences in distributions of age may, in part, explain the lack of consistent findings in these studies.

In summary, comparing all these clinical-prognostic variables, we found that Ki67 index was the main prognostic factor associated with survival ($P = 0.001$), but the overexpression of p53, EGFR and PDGF was not. Multiple factors, including age, extent of resection and location of the lesion, complicate the analysis of these as prognostic variables.

While much work remains to develop biomarkers that can reliably predict response among malignant glioma patients, one clearly important modulator of temozolomide response is O6-alkylguanine-DNA alkyltransferase (AGT). AGT is a critical DNA repair protein, also referred to as O6-methylguanine-DNA-methyltransferase, which removes chloroethylation or methylation damage from the O⁶ position of DNA guanines, thereby protecting normal cells from exogenous carcinogens, and similarly protecting tumor cells from alkylating and methylating chemotherapeutic agents. AGT levels vary significantly across and within tumor types. Methylation of CpG islands within the AGT promoter is an epigenetic factor that can diminish AGT transcription and expression. AGT levels can be most readily measured by immunohistochemistry or by a methylation-specific polymerase chain reaction assay. Reardon *et al*⁵³ initially implicated AGT in temozolomide responsiveness among malignant glioma patients. In a series of 36 newly diagnosed patients, including 33 with GBM, the response rate to temozolomide was 60% among patients with low-level AGT (detected by immunohistochemistry in <20% of cells) compared to only 9% among patients with high-level AGT (present in >20% of tumor cells). Similarly, following carmustine (BCNU) chemotherapy, Esteller⁵⁴ noted a radiographic response in 12 (64%) of 19 malignant glioma patients with methylated AGT compared with only 1 (4%) of 28 patients with unmethylated AGT. Two subsequent, prospective studies demonstrated that AGT methylation is associated with better survival among malignant glioma patients treated with temozolomide. The studies suggested that tumor AGT status is an independent predictor of outcome following therapy with alkylator-based chemotherapy, because AGT status does not correlate with established clinical prognostic factors. Controversy exists regarding the optimal technique to assess tumor AGT status. Validated, commercially available, polymerase chain reaction-based AGT methylation assays are not yet approved for clinical use and may be technically challenging. Immunohistochemical techniques are more widely available, but may be less reliable since external factors such as glucocorticoids, radiation therapy, and genotoxic agents can influence AGT expression⁵³. Therefore, pending validation of AGT testing in additional, prospective GBM clinical trials and the development of a validated, commercially available assay, it is premature to routinely incorporate AGT status in directing GBM therapy.

Conclusions

Efforts to understand why some patients live longer or shorter than the average may provide insights into the biology of these neoplasms. Nowadays, the most consistent predictor of survival in malignant gliomas is patient age at diagnosis. Multiple studies have shown that younger patients affected by these tumors live longer following the initial diagnosis, even after adjust-

ing for histological grade, tumor size, KPS, extent of resection, and treatment following biopsy/resection. Despite falling within a single histological grade, glioblastomas exhibit significant genetic heterogeneity. It is likely that these genetic differences at least partially account for the differences in survival time among patients with these neoplasms.

Efforts have been made by many groups to test whether the genetic alterations found in these tumors can, in addition to patient age, predict prognosis. The most common alterations in high-grade astrocytic gliomas include mutations in the p53 gene, amplification and rearrangement of the EGFR gene, and amplification of a region of chromosome 12q that encodes murine double minute 2 and cyclin-dependent kinase 4²⁶. Many of these alterations have been tested for potential as prognostic markers. However, there has been no consensus for the prognostic value of most of these

commonly altered genes in GBM. For example, some investigators have found that gliomas with the p53 gene mutation or immunopositivity (as a marker of abnormality in the p53 pathway) predict shorter survival²⁷⁻²⁹, whereas others found no statistically significant relationship^{5,39,40,49-51}. In addition, one recent study showed that mutation in p53 predicted longer survival in patients with GBM⁵².

The results of the present study suggest that an age of 60 years or older ($P < 0.03$), postoperative KPS ≤ 70 ($P = 0.04$), non-total tumor resection ($P = 0.03$), tumor size > 4 cm ($P = 0.01$) and proliferation index overexpression ($P = 0.001$) were associated with a poor prognosis. p53, GFAP and EGFR overexpression were not significant in this analysis. Nevertheless, additional studies are likely to further highlight the genetic complexity of specific prognostic markers of this morphologically defined entity.

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