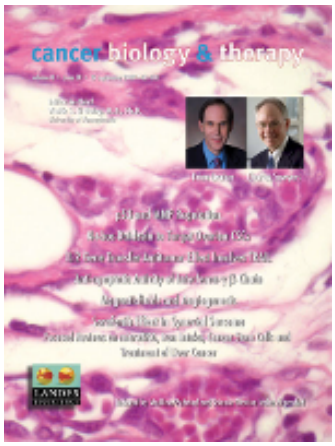

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Clinical Study

Survival of high grade glioma patients depends on their age at diagnosis

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Key words: brain tumors, glioblastoma, micronuclei, survival, prognosis, age, hazard

Background: Although the prognosis for malignant gliomas is normally dismal, it's not infrequent in neurooncologist's experience to find cases with unusually prolonged survival. In order to understand what factors influence survival of high grade glioma patients, a cohort of 196 high (III-IV) grade glioma patients was investigated for possible association between (1) survival and age at diagnosis; (2) survival and micronuclei in tumor tissue; (3) survival and gender; (4) micronuclei in tumor tissue and age at diagnosis.

Results: Patients diagnosed at an older age (>64 years) had a significantly higher hazard as compared to younger patients (≤64 years), indicating that older patients survived shorter. On the contrary, no association was found between survival and micronuclei or gender.

Methods: Survival analysis was performed by the Cox' proportional hazards regression model.

Conclusions: Age at diagnosis, together with other established prognostic factors such as histologic characteristics, extent of surgery and Karnofsky Performance Score may to a certain extent predict survival of high grade glioma patients.

Introduction

Gliomas are the most common form of primary brain tumors accounting for >70% of new cases of primary malignant central nervous system (CNS) tumors in western countries (reviewed in ref. 1). Gliomas are classified into low- and high-grade types according to their morphological features. Cells of low-grade (WHO grade I and II) gliomas are well differentiated with clear histological similarity to astrocyte or oligodendrocyte lineage. High-grade gliomas [anaplastic astrocytomas (WHO grade III) and multiform glioblastomas (GBM—WHO grade IV)] are more anaplastic with features resembling immature astrocytes, oligodendrocytes or a mixture of both types. Compared with lung and breast cancer, which have rates of about 60 per 100,000 in western countries, the rate of gliomas is ~tenfold lower. Nevertheless these cancers are associated with disproportionately high morbidity and mortality the median survival being only 12–15 mo for patients with GBM and 2-5 y for patients with anaplastic gliomas.[1-3] Further, not only do glioma patients have to cope with the diagnosis of incurable disease, they and their families are usually also confronted with the patient's decrease in cognitive and emotional function as a result of cerebral disease. Although the prognosis for malignant gliomas remains dismal, it's not infrequent in neurooncologist's experience to find cases with prolonged survival of six or more years.[4,5] There is a need to understand what factors influence survival of high grade glioma patients. Using a cohort of 196 high grade glioma patients we have investigated the possible association between (1) survival and age at diagnosis; (2) survival and micronuclei (MN) in tumor tissue; (3) survival and gender; (4) MN in tumor tissue and age at diagnosis.

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Results

Figure 1 shows Kaplan-Meier survival curves of high grade glioma patients as a function of age at diagnosis. The estimated median survival time (MST) was 394 and 273 days, respectively in patients aged ≤64 and >64 y at the time of diagnosis of malignant glioma (Table 1). Age at diagnosis was significantly associated with survival [hazards ratio (HR) = 1.74, 95% confidence interval (CI) = 1.29, 2.35, $p < 0.001$] indicating that patients diagnosed at a young age (≤64 y) survived longer than those diagnosed at older ages. MST was 340 d in the subgroup of patients with a MN frequency ≤5.4/1,000 cells and 321 d in glioma patients with a frequency of MN >5.4/1,000 cells (Table 1). MST was similar in males and females (321 and 339 d, respectively—Table 1). Neither MN frequency nor gender were associated to survival ($p = 0.50$ and 0.80, respectively). MN frequency did not correlate with age at diagnosis indicating that chromosomal instability of high grade glioma tumors is not dependent on patients' age (Spearman $r = 0.028$; Fig. 2).

Discussion

A number of genetic alterations has been described in high grade gliomas suggesting that genetic instability may play a role in this disease (reviewed in ref. 1). MN can be generated as a consequence of chromosome breakage and/or chromosome loss (aneuploidy) and their frequency is a biomarker of cancer risk that integrates acquired mutations and genetic susceptibility.[9] The combination of different loci involved in genetic instability and their interaction with genotoxic agents may modulate MN induction.[10] However, whether MN frequency represents an early biomarker for cancer progression still is an open question.[11] A number of studies have addressed the possible correlation between MN formation and cellular sensitivity to radio/chemotherapy in high grade gliomas. MN frequency correlated with cell proliferation rate and DNA damage induced by radio/chemotherapy but not with induction of apoptosis.[12-14] To our knowledge, this is the first study on the possible prognostic value of MN frequency in untreated high grade gliomas. MN frequency was not associated to survival nor to age at diagnosis. Hence, despite MN frequency may correlate with cancer progression in some cases,[15,16] it has no prognostic value in high grade glioma. On the contrary, age at diagnosis is associated to survival in glioma patients. Young age and high Karnofsky Performance Score (KPS) have been associated with prolonged survival time for patients with Grade III or IV gliomas in previous studies[17-19] although no statistically significant correlation between age and survival has been reported as well.[20] In a cohort of 196 grade III-IV glioma patients, we have found using the proportional hazards regression model[8] that patients diagnosed at an older age (>64 years) have a significantly higher hazard as compared to younger patients (≤64 years), indicating that older patients survive shorter. Hence age at diagnosis, together with other established prognostic factors such as histologic characteristics, extent of surgery and KPS may to a certain extent predict survival of high grade glioma patients. It is clear that more precise prognostic factors are required in order to improve our knowledge of disease progression and orientate therapies.

Methods

Patients. Patients were recruited at Azienda Ospedaliera S. Martino, Genova, Italy and Azienda Ospedaliera S. Corona, Pietra Ligure, Savona, Italy over a period of 16 y, ranging from 1992–2008. Patients were not pre-treated with radio- or chemotherapy before surgery. All patients had a confirmed diagnosis of anaplastic astrocytoma (WHO grade III; n = 22), multiform glioblastoma (WHO grade IV; n = 133) or tumors with both components (WHO grade III-IV; n = 41).

Micronuclei analysis. Tumor specimens were fixed in formalin. Paraffin-embedded sections were rehydrated and stained with 50% GIEMSA for 5 min. They were then dehydrated with ethanol and isopropanol, cleared and assembled. MN were scored by sight under a light microscope (3,000 cells/slide).[6]

Statistical analysis. The Kaplan-Meier survival function was used to describe the survival times of the study population.[7]

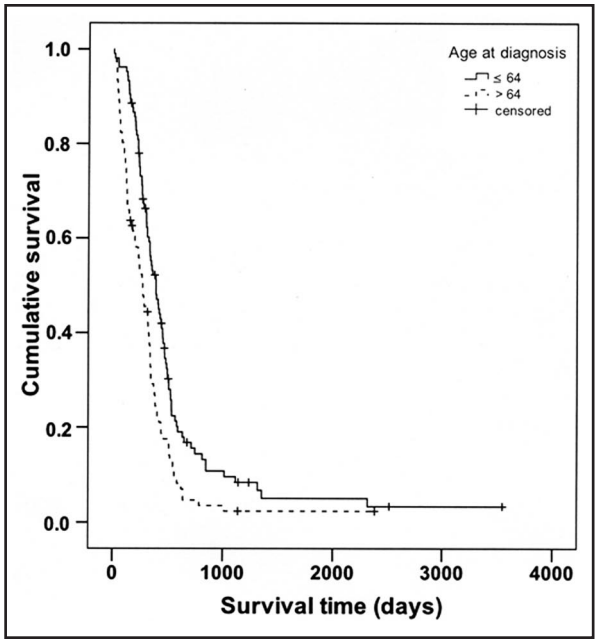

Figure 1. Kaplan-Meier survival curves of high grade glioma patients as a function of age at diagnosis. The estimated median survival time was 394 and 273 d, respectively in patients aged ≤64 and >64 y at the time of diagnosis of malignant glioma. Age at diagnosis was significantly associated with survival (Hazard ratio = 1.74, 95% confidence interval = 1.29, 2.35, p < 0.001).

Table 1 **Distribution of the study population by age at diagnosis, frequency of micronuclei and gender and the associated Kaplan-Meier estimated median survival time, hazard ratio and 95% confidence interval estimated by using Cox' proportional hazards regression model**

Covariate	No.	MST[a] (days)	HR[a]	95%CI[a]	p
Age at diagnosis					<0.001
≤64 y	105	394	1		
>64 y	91	273	1.74	1.29, 2.35	
MN[a]					0.50
≤5.4/1,000 cells	99	340	1		
>5.4/1,000 cells	97	321	1.11	0.83, 1.49	
Gender					0.80
Females	80	339	1		
Males	116	321	0.96	0.71, 1.30	

[a]MST, median survival time; HR, hazard ratio; CI, confidence interval; MN, micronuclei.

Survival time, the response variable measuring the duration of time until death, was defined as the time between the date of cancer diagnosis and the date of death and was expressed in days. The association between survival time and the frequency of MN, age at diagnosis, and gender was assessed by using the proportional

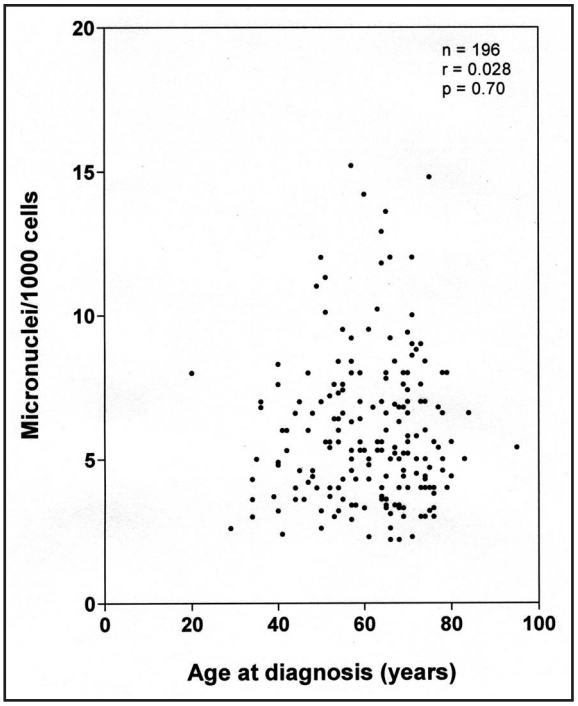

Figure 2. Scatterplot of micronuclei frequency (micronuclei/1,000 cells) as a function of age at diagnosis (years). The correlation between micronuclei frequency and age determined by Spearman rank correlation test was not statistically significant (p = 0.70).

hazards regression model.[8] Patients still alive at the end of follow up were considered censored. Patients were cathegorized into two groups of equal size based on their median age at diagnosis (64 y) and the median level of MN in tumoral tissue (5.4 per 1,000 cells). The cathegorized covariates were assigned the value 0 (zero) for observed values ≤ the median value and 1 for observed values > the median value. Comparisons were considered to be statistically significant at a probability (p) level <0.05. The relationship between the frequency of MN and age at diagnosis was investigated using the non-parametric Spearman rank correlation test. The frequency of MN measured in males and females was compared using the Student's test. Statistical analyses were conducted using statistical software Prism 5 by GraphPad and SPSS (SPSS Release 17, 2009).

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