



Prognostic Importance of Ki-67 Labeling Index in Grade II Glial Tumors

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OBJECTIVE

To date, several methods have been identified for predicting the prognostic subgroups of grade II gliomas; however, these methods have some limitations in predicting survival. So, we aimed to determine the predictive role of Ki-67 labeling index (LI) on survival.

METHODS

Between 1995 and 2014, patients with grade II gliomas were retrospectively analyzed. All patients received radiotherapy (RT).

RESULTS

This study included 78 patients with median 44 (range, 6–137) months follow-up. Patients aged ≥ 40 years had a poorer overall survival (OS) than those aged < 40 years ($p=0.04$). Patients with gross total resection/subtotal resection had a longer OS than those with biopsy/partial resection ($p=0.001$). If the disease had recurrence or progression during the follow-up period, the patients had a poorer OS ($p=0.01$). Patients with a Ki-67 LI $\geq 4\%$ had a poorer OS than those with Ki-67 LI $< 4\%$ ($p=0.001$). The extent of resection, recurrence, or progression, and Ki-67 $\geq 4\%$ were the independent prognostic factors for OS.

CONCLUSION

In our opinion, Ki-67 LI is an important prognostic factor for grade II gliomas, but it cannot be used as a diagnostic measure alone. It must be used in combination with the other prognostic factors.

Keywords: Grade II glial tumors; Ki-67 labeling index; Prognostic factors; Radiotherapy; Survival.

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Introduction

Low-grade gliomas (LGGs) account for 15%–20 % of all primary brain tumors. LGGs may eventually undergo malignant transformation and progress into high-grade gliomas, resulting in high morbidity and mortality. LGGs harbor a heterogeneous spectrum of histopatho-

logical subtypes, and therefore, optimal treatment is controversial.[1-3] To date, several methods for predicting the prognostic subgroups of LGGs have been identified. These methods evaluated clinical variables [4,5], tumor grading [6], and histopathologic classification. [6] Tumor grading and histopathologic classification are subjective methods since they are based on light micro-

scopic techniques.[7] Besides, almost all methods for predicting the prognostic subgroups of LGGs have some limitations in predicting survival. In this regard, there is a need to develop new prognostic markers with the aim of predicting tumor behavior and prognosis.

Proliferative phases of the cell cycle are composed of G1-phase, S-phase, G2-phase, and M-phase. The Ki-67 monoclonal antibody is detected during all these proliferative phases of the cell cycle, but absent in the resting cells (G0-phase).[8] Ki-67 positive cells can be immunohistochemically imaged, identified, counted, and labeled as a percentage of total cells. This relationship is named the “labeling index” (LI) or the “proliferation index.” Since its discovery in the 1980s, there has been an increasing interest in the role of Ki-67 index as a proliferation marker in cancer, and it has been shown to be clinically useful in distinguishing the tumor behavior and correlates well with histologic grade, mitotic activity, and other proliferation markers.[9-18] In this current study, we aimed to investigate the possible prognostic relationship between Ki-67 LI and the survival outcomes of adult patients with World Health Organization (WHO) grade II glial tumors who were treated with radiotherapy (RT).

Materials and Methods

2.1. Patient population

Between 1995 and 2014, adult patients with grade II glial tumors who had been irradiated at our departments were evaluated in this retrospective study. Eligible patients were required to have histopathologically proven grade II glial tumors, be over 16 years of age, and have their paraffin-embedded tumor tissue blocks available. The study was approved by the review boards and conducted according to the ethical principles of the declaration of Helsinki.

2.2. Radiotherapy

Radiotherapy is used in patients with tumors that cannot be totally removed or in patients with high-risk features.[19] High-risk factors were defined by Pignatti and colleagues; these factors were identified as age ≥ 40 years, tumor size ≥ 6 cm, tumor crossing the midline, preoperative neurologic deficit existence, and astrocytoma histology subtype. The presence of ≥ 3 of the above-mentioned factors is defined as high risk.[20] Postoperative early RT is frequently used in patients with WHO grade II glial tumors that cannot be totally resected or in patients who are suspected to have high-risk features, whereas delayed RT is frequently used in patients with recurrence or progression. The patients received RT with 1.8–2 Gy

fractions 5 days a week for a total dose of 50–66 (mean, 54 Gy) Gy to the tumor (with 1–2 cm margins).

2.3. Histopathology and Ki-67 expression

Two experienced neuropathologist retrospectively re-defined all tumors based on the 2007 WHO classification system for brain tumors.[21] If the neuropathologist did not agree on the definition of the pathological status, a third neuropathologist re-evaluated the tumor tissue. Immunohistochemical staining for Ki-67 was performed on formalin-fixed, paraffin-embedded tissue sections. Proliferation indexes were reported as the percentage of tumor cell nuclei labeled with the Ki-67 monoclonal antibody in paraffin-embedded specimens. For each patient, areas with the highest number of positively stained tumor nuclei were chosen for reporting.

2.4. Clinical evaluation and follow-up

Following RT, patients were followed up every 3 months for 2 years, every 6 months for 3–5 years, and annually thereafter.

2.5. Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences software, v 13.0 (SPSS, Chicago, IL, USA). Patient, disease, and treatment characteristics were analyzed using descriptive statistics. The median value, mean value, proportion value, ranges, and standard deviation values were reported. Categorical variables were compared using Pearson's Chi-square test, and continuous variables were compared using independent samples t-test and ANOVA test. Overall survival (OS) time was defined as the time from diagnosis to the date of the death or last follow-up. Progression-free survival (PFS) was defined as the time from diagnosis to the date of documented progression or recurrence. Survival analyses were performed using the Kaplan–Meier method, and subgroups were compared using the two-sided log rank test. Cox proportional hazard regression analysis was used for the estimation of hazard ratios and 95% confidence intervals (CIs). Variables with statistical significance ($p \leq 0.05$) in univariate analysis were included as covariates in multivariate analysis. A two-sided p-value of ≤ 0.05 was considered to be statistically significant.

Results

3.1. Patient and tumor characteristics

This study included 78 patients with grade II glial tumors with a median follow-up of 44 (range, 6–137 months)

Table 1 Patient, tumor, and treatment characteristics		
Variables	No. of patients (total:78)	%
Age (years)		
Median	38	
Range	16-62	
<40	44	56
≥40	34	44
Gender		
Male	43	55
Female	35	45
Surgery type		
Biopsy or partial resection	18	23
Subtotal resection	35	45
Gross total resection	25	32
Histopathology		
Grade II astrocitoma	59	76
Grade II oligodendroglioma	15	19
Grade II oligoastrocitoma	4	5
Neurologic deficit existence at diagnosis		
No	43	55
Yes	35	45
Seizures at diagnosis		
No	44	56
Yes	34	44
Timing of RT		
Early	51	65
Delayed	27	35
Radiation dose (Gy)		
Median	54	
Range	50-66	
Chemotherapy		
No	69	88
Yes	9	12
Ki-67 index (%)		
Median	1.5	
Range	0-40	
<4	67	86
≥4	11	14

months. Of these patients, 59 had been diagnosed with astrocytomas, 15 with oligodendrogliomas, and four with oligoastrocytomas. Patients, tumor, and treatment characteristics are summarized in Table 1.

3.2. Treatment characteristics

Surgery was the initial treatment approach for all patients. Gross total resection (GTR) was performed in 25 patients (32%), subtotal resection (STR) in 35 (45%), partial resection (PR) in six (8%), and only biopsy (Bx) in 12 (15%). Postoperatively, 51 patients (65%) received adjuvant early RT, while 27 patients (35%) received ad-

Table 2 Univariate cox proportional hazard regression analysis related with OS			
Variables	HR	95% CI	P-value
Age			
<40 years	1		
≥40 years	2.34	0.98-5.59	0.056
Extent of removal at diagnosis			
GTR or STR	1		
Bx or PR	4.81	1.67-13.87	0.004*
Recurrence or progression			
No	1		
Yes	5.56	1.29-10.23	0.02*
Diagnose with biopsy only			
No	1		
Yes	4.96	1.67-14.68	0.004*
Ki-67 index			
<4 %	1		
≥4 %	4.49	1.76-11.47	0.002*

Abbreviations: OS, overall survival; GTR, gross total resection; STR, subtotal resection; PR, partial resection; Bx, biopsy.
* Statistically significant.

juvant delayed RT. The median radiation dose was 54 (range, 50-66) Gy.

3.3. Ki-67 status

Jaros et al. reported that the heterogeneity in Ki-67 LI was around 20% astrocytomas so that the average values of Ki-67 LI represent the proliferative potential of the tumor more than the maximal Ki-67 value.[22] Otherwise, Fisher et al. found that the average Ki-67 value was much more associated with survival than the maximal value of Ki-67.[23] For this reason, the average Ki-67 value was used for the analysis. Seventy-two patients (92%) were Ki-67 positive (i.e., the percentage of Ki-67 staining cells was >0). The median Ki-67 LI value was 1.5% (range, 0-40). The majority of patients (51%) had a Ki-67 LI value of ≤2%. Eleven patients (14%) had a Ki-67 LI value of ≥4% and eight (10%) had a Ki-67 LI value of ≥5%. Only one patient had a Ki-67 LI value of ≥10%. Survival analyses were performed using the Kaplan-Meier method at various cutoff points for average Ki-67 value at 1% intervals between 0% and 10%, and the average Ki-67 LI value of ≥4% was associated with OS.

3.4. Survival analysis

The median follow-up time was 44 (range, 6-137) months. Fifty-six patients (72%) were alive and 20 of them had a disease when the survival analysis was performed. The mean OS was 88 (range, 73-102) months. The 2-, 5-, and 10-year OS rates were 90%, 70%, and

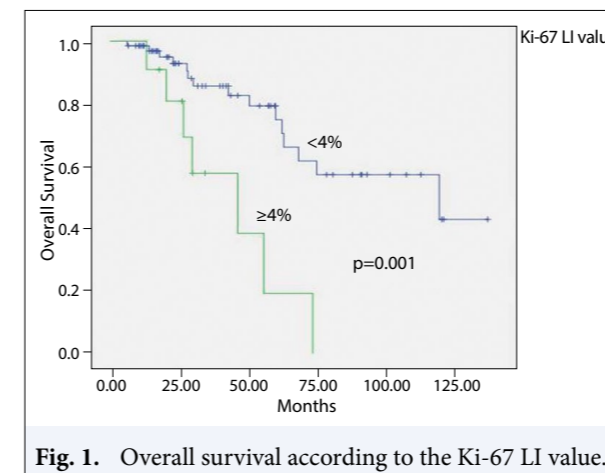


Fig. 1. Overall survival according to the Ki-67 LI value.

40%, respectively. The extent of resection, recurrence, or progression and average Ki-67 LI value were the significant prognostic factors for OS. A trend of higher survival was found in patients aged <40 years. The results of univariate analysis for OS are summarized in Table 2.

Patients with a Ki-67 LI value of ≥4% had a poorer OS than patients with a Ki-67 LI value of <4% (p=0.001). The mean OS was 96 months for patients with a Ki-67 LI value of <4% versus 43 months for the patients with a Ki-67 LI value of ≥4% (Fig 1). The mean Ki-67 LI value was 1.8% in patients who were alive versus 4.7% in patients who died of disease (p=0.01).

The mean PFS was 67 (range, 56-77) months for all the patients. The 2-, 5-, and 10-year PFS rates were 85%, 49%, and 12%, respectively. The average Ki-67 LI was not associated with PFS. The extent of resection was the only independent prognostic factor that affected PFS in survival analysis.

Discussion

Ki-67 LI was presented more than two decades ago as a measure of tumor proliferation and because of the need for a prognostic marker that might help clinicians to direct therapy and predict tumor behavior and prognosis [9-18]. Many researchers demonstrated that the Ki-67 expression as a cell division marker has been associated with radioresistance in some cancers.[24] Inhibition of apoptosis could be involved in the complex mechanism of radioresistance with prognostic implications.[25-27] Contrary to these opinions, some researchers claimed that the proliferative cells have relative radiosensitivity compared with non-proliferating cells.[28] A higher Ki-67 LI theoretically defines more

proliferative tumors; therefore, it may be expected that patients with higher Ki-67 index might be associated with higher radiosensitivity.[23]

In the current study, we aimed to investigate the possible prognostic relationship between Ki-67 LI and the outcomes of adults with grade II glial tumors who were treated with RT. According to our results, age, extent of resection, recurrence or progression, and average Ki-67 LI value were the significant prognostic factors for OS. The average Ki-67 LI value of ≥4% was associated with OS. Patients with a Ki-67 LI value of <4% PFS was not affected by Ki-67 LI according to our results. This can be explained by the fact that PFS may have been more influenced by other prognostic factors, such as variability of surgery and variability in the diagnosis of progression.

Despite numerous studies, there are questions about the impact of Ki-67 LI on survival.[7,23,28-32] Some authors have reported correlations between Ki-67 LI and survival.[7,23,29-7] The others did not show an association between Ki-67 LI and survival outcomes.[30,7] These different results can be explained by the variety of techniques that evaluate Ki-67 LI, subjectivity of interobserver, and heterogeneity of Ki-67 level within the specimen.[12] Some studies included limited number of patients and histologically heterogeneous patient populations. Mckeever et al. reported a strong correlation between low Ki-67 LI and longer survival in 50 patients with WHO grade II astrocytomas.[29] Fisher et al. showed in their research that an average Ki-67 index of >5% was predictive of cause-specific survival (CSS) and that a maximal Ki-67 index of >2% indicated a poorer CSS within the delayed RT subgroup.[23] Similarly, Montine et al. revealed that patients with a Ki-67 LI ≥3% had a poorer survival than patients with a Ki-67 LI of <3%.[13]

Although most of the studies showed a significant increase in Ki-67 LI with the increasing grade of astrocytomas, Ki-67 LI values overlapped in some studies. [31] The Ki-67 LI value of glioblastoma can be as low as those for LGGs, indicating that the Ki-67 LI value cannot be used as a diagnostic measure alone [33] and must be used in combination with other prognostic factors.

According to our results, the other independent prognostic factor that affected OS was recurrence or progression of disease. Our current study revealed that the avoidance of local recurrence or progression after surgery was of comparable relevance to mortality. Local recurrence or progression of disease after surgery can be included as a risk factor predicting decreased

OS for WHO grade II gliomas. There is a need for prospective studies for the clarification of this issue.

WHO classification of central nervous system tumors updated in 2016, which compose genotypic and phenotypic parameters. The absence of re-classification of tumors according to new version of WHO staging and the retrospective feature of the study were the major limitations of study.

Conclusion

WHO grade II gliomas are a heterogeneous group of tumors, and there is still significant disagreement between clinicians concerning the optimal treatment. Prognostic factors are important for the prediction of survival and for deciding the most appropriate treatment modality. According to our current study, besides the extent of surgery and recurrence or progression, Ki-67 LI $\geq 4\%$ was an independent prognostic factor predicting OS. Although Ki-67 LI is a reliable prognostic factor for grade II glial tumors, it should not be used as a diagnostic measure alone, but in combination with other prognostic factors.

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