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 ≥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 ≥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 other prognostic factors.

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

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 >2 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 redefined 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 Ki-67 positive cells.

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, 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≤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)
months. Of these patients, 59 had been diagnosed with astrocytomas, 13 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 adjuvant delayed RT. The median radiation dose was 54 (range, 50–66) Gy.

#### 3.3. Ki-67 status

Jaroš 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 presented more than two decades ago as a measure of tumor proliferation and because of the Ki-67 expression as a cell division marker has been related with OS.[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% had a poorer OS than 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.[7,23,29,37] 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 <3% 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

### Table 1. Patient, tumor, and treatment characteristics

<table>
<thead>
<tr>
<th>Variables</th>
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</tr>
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<tr>
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</tr>
<tr>
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<td>Range</td>
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<tr>
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</tr>
<tr>
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<tr>
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<tr>
<td>Timing of RT</td>
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<td>51</td>
</tr>
<tr>
<td>Delayed</td>
<td>27</td>
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<tr>
<td>Radiation dose (Gy)</td>
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<td>Range</td>
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</tr>
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<td>Ki-67 index (%)</td>
<td></td>
</tr>
<tr>
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<tr>
<td>Range</td>
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### Table 2. Univariate cox proportional hazard regression analysis related with OS

<table>
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<th>Variables</th>
<th>HR</th>
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<th>P-value</th>
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<tr>
<td>≥40 years</td>
<td>2.34</td>
<td>0.98-5.59</td>
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<td></td>
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<td>GTR or STR</td>
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<tr>
<td>Bx or PR</td>
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<td>1.67-13.87</td>
<td>0.004*</td>
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<tr>
<td>Recurrence or progression</td>
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<td></td>
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<tr>
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<td>1</td>
<td></td>
<td></td>
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<td>Yes</td>
<td>5.56</td>
<td>1.29-10.23</td>
<td>0.02*</td>
</tr>
<tr>
<td>Ki-67 index &lt;4%</td>
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<td></td>
<td></td>
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<tr>
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<tr>
<td>Yes</td>
<td>4.96</td>
<td>1.67-14.68</td>
<td>0.004*</td>
</tr>
<tr>
<td>Ki-67 index ≥4%</td>
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<td></td>
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<tr>
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<tr>
<td>≥40 years</td>
<td>4.49</td>
<td>1.76-11.47</td>
<td>0.002*</td>
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</tbody>
</table>

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

*Statistically significant.

#### 3.4. Survival analysis

The median follow-up time was 51 (range, 6-137) months. Fifty-six patients (72%) were alive and 20 of these patients, 51 had been diagnosed with astrocytomas, 15 with oligodendrogliomas, and four with oligoastrocytomas. Patients, tumor, and treatment characteristics are summarized in Table 1.

The median radiation dose was 54 (range, 50–66) Gy.

### Table 2. Univariate cox proportional hazard regression analysis related with OS

<table>
<thead>
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<th>Variables</th>
<th>HR</th>
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<th>P-value</th>
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<tr>
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<td>&lt;40 years</td>
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<td>GTR or STR</td>
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<td>Bx or PR</td>
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<td>Recurrence or progression</td>
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<td>Ki-67 index &lt;4%</td>
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<td>4.49</td>
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</tr>
</tbody>
</table>

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

*Statistically significant.

#### 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 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]
OS for WHO grade II gliomas. There is a need for pro-
spective studies for the clarification of this issue.

WHO classification of central nervous system tu-
mors 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 tu-
mors, and there is still significant disagreement between
clinicians concerning the optimal treatment. Prognostic
factors are important for the prediction of survival and
deciding for the most appropriate treatment modality.
According to our current study, besides the extent of sur-
gery and recurrence or progression, Ki-67 LI ≥24% was an
independent prognostic factor predicting OS. Although
Ki-67 LI is a reliable prognostic factor for grade II glial
neoplasms, it should not be used as a diagnostic marker
alone, but in combination with other prognostic factors.

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Conflict of Interest: The authors declare that there is no con-
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G.K., E.Y.E.; Supervision – G.K., E.Y.E., H.S.E.; Materials –
G.K., E.Y.E., H.S.E., H.O., M.A., M.K., H.B., E.K.; Data col-
Analysis and/or interpretation – G.K., E.Y.E., H.S.E.; Litera-
ture search – G.K., E.Y.E.; Writing – G.K., E.Y.E., H.S.E.; Criti-
cal review – G.K., E.Y.E., H.S.E.

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