Expression of Ki-67 in Low-Grade and High-Grade Astrocytomas. A Literature Review.

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ABSTRACT

OBJECTIVE. This review aims to carry out a survey on the importance of Ki67 in the astrocytomas study. METHODS. A search in the electronic database of Medline via PubMed and using MESH terms was carried out. Articles were published between January 2005 and December 2015. All studies were analyzed by two experienced researchers, using inclusion and exclusion criteria for the selection of studies. RESULTS. Five studies showed an association between cell proliferation and survival time. Four articles mentioned as cut-off point for survival a Ki67 of 10% and a fifth article a Ki67 of 14.3. A study showed an association between therapeutic failure and Ki67. Four studies have made the association between Ki67 and World Health Organization classification. CONCLUSION. High levels of Ki67 are associated to high grade astrocytomas and lower survival time.

Key words: Astrocytoma; Glioma; Glioblastoma; Prognosis; Mortality

RESUMO


Palavras-chave: Astrocitoma; Gliomas, Glioblastoma; Prognóstico; Mortalidade

INTRODUCTION

Gliomas are tumors that originate in glia cells, and according to the cell of origin they are called astrocytoma, oligodendroglialoma, oligoastrocytomas and ependymomas. They are the most common primary neoplasms in the central nervous system (CNS), representing approximately 70-80% of all cases.1,2,3,23

The main risk factors are exposure to ionizing radiation and some rare genetic conditions such as neurofibromatosis and tuberous sclerosis.5,6

These tumors can present a variety of neurological manifestations, such as epilepsy, motor deficits or sensory and behavioral changes, for example. The complete surgical removal usually is not possible due to their infiltrative characteristic or its location in eloquent areas.7

Incidence has been increasing over the past 21 years and this growth might be justified by the greater knowledge of the population and physicians about the disease and access to imaging examinations, allowing a greater number of diagnoses, including tumors which are still asymptomatic.7

The astrocytomas are more prevalent gliomas and according to the classification of the World Health Organization (WHO) can be of low or benign grade (grade I and II) and high-grade or malignant tumors (Grade III and IV) taking into account
the histopathological criteria: nuclear atypia, mitosis, cell proliferation and presence of necrosis.

The high grade astrocytomas are the most common primary malignant neoplasms of the CNS in adults, which even adequately treated with surgical resection, chemotherapy and radiation therapy, the prognosis is reserved. However, the survival improvement seems to depend on the knowledge and manipulation of the pathways that regulate the growth of these aberrant tumors.

The WHO classification has some limitations in predicting survival, and there is a need for additional methods of prognosis. The monoclonal antibody anti-Ki67 can play this role, since it recognizes an existing protein in proliferating cells and absent in quiescent cells, being a good marker of cell proliferation.

Several studies point to a substantial increase in cell proliferation with the increase of the malignancy grade of the tumor, making the anti-Ki67 a potential biomarker for prognosis and survival in astrocytomas.

The aim of this review is to perform a survey on the importance of the Index of cell proliferation measured by Ki67 in the diagnosis, classification and prognosis of astrocytomas.

**Material and Methods**

It was carried out a research in the electronic database of MEDLINE via PubMed, on the index of cell proliferation measured with the anti-Ki67 in astrocytomas. Only articles published between January 2005 and December 2015. The researched terms were the MESH terms: (antigen Ki67” OR "MIB1 protein, human) AND (astrocytoma” OR "glioma” OR “glioblastoma”).

The inclusion criteria were articles: a) written in English, b) that assess the rate of cell proliferation in astrocytomas by means of a marker anti-Ki67, c) that evaluate the rate of cell proliferation in astrocytomas by means of a marker anti-MIB1, d) use the anti-Ki67 and or anti-MIB1 as a diagnostic criterion, and use the anti-Ki67 and or anti-MIB1 as prognostic marker and f) use the anti-Ki67 and or anti-MIB1 as a marker of survival.

All papers were inspected by two experienced authors to exclude: a) duplicated articles, b) access only to the abstract, c) case reports or case series, d) editorial e) remarks, f) reviews of the literature, g) Letters to the editor, h) articles which referred to other histological types rather than astrocytomas and i) studies that used the anti-Ki67 or anti-MIB1 as a method of comparison of the effectiveness of other biomarkers.

**Results**

Selected studies

Out of the 370 studies found in MedLine using keywords, only 33 met the inclusion criteria. Out of these, 23 articles were excluded, because two of them were duplicated, three only the abstract was accessed, eight were non-astrocytomas tumors, three were reviews of the literature and seven articles used the anti-Ki67 or anti-MIB1 as a method of comparison to evaluate the effectiveness of other biomarkers (Figure 1).

**Studies characteristics**

Five studies showed an association between the rate of cell proliferation with the Ki67/MIB1 and the survival time of the patients. The higher the rate, the lower is the survival. In one of these, the analysis was performed with recurrent astrocytomas (Table 1).

Four articles cited as the cut-off point for survival a Ki67 of 10%, and patients with lower score, showed a higher survival and patients with values greater than 10% showed lower survival (Table 1).

One article mentioned as the cut-off point for survival a Ki67 of 14.3%, and patients with lower score showed a higher survival and patients with values greater than 14.3% showed lower survival (Table 1).
A study showed an association between the therapeutic failure and cell proliferation rate. The higher the Ki67/MIB1, the lower is the treatment effectiveness (Table 1).

Four studies made the association between cell proliferation and the WHO histopathological classification. For data, all of them there were statistically significant differences in Ki67/MIB1, between gliomas of low and high grade (Table 1).

Two studies found no statistically significant differences in Ki67/MIB1 between gliomas grade I and grade II. One study found no statistically significant difference in Ki67/MIB1 between gliomas grade III and IV (Table 1).

Table 1. Summary of features found in the selected studies.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Journal</th>
<th>Country</th>
<th>Important Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habbenstal et al.</td>
<td>2011</td>
<td>Diagnostic Pathology</td>
<td>Norway</td>
<td>Patients with anaplastic astrocytomas and Ki67 &lt; 10% have statistically higher survival time and lower Ki67/MIB1 in the Kaplan-Meier curve than those with a higher rate of 10%, statistically significant difference.</td>
</tr>
<tr>
<td>Uehara et al.</td>
<td>2013</td>
<td>Oncol Lett</td>
<td>Japan</td>
<td>A high rate of cell proliferation (Ki67) is strongly associated with a higher degree of therapeutic failure in patients with glioblastoma multiform.</td>
</tr>
<tr>
<td>Chen et al.</td>
<td>2014</td>
<td>J Clin Oncol</td>
<td>India</td>
<td>The Ki67 expression increased significantly with the gliomas malignancy grade. A statistically significant difference was observed among all WHO histological grade.</td>
</tr>
<tr>
<td>Silva et al.</td>
<td>2014</td>
<td>Int J Clin Exp Pathol</td>
<td>Norway</td>
<td>The values of anti-Ki67 were similar between gliomas grade I and II and between the gliomas grade III and IV, more statistically different between low-grade (III) and high-grade (IV) gliomas.</td>
</tr>
<tr>
<td>Saha et al.</td>
<td>2014</td>
<td>Indian J Med Paediatr Oncol</td>
<td>India</td>
<td>Patients with astrocytomas and Ki67 &gt; 14.3 had lower survival time and cumulative survival in 2.5 years than patients with lower rates of cell proliferation.</td>
</tr>
<tr>
<td>Zeng et al.</td>
<td>2015</td>
<td>Oncotarget</td>
<td>China</td>
<td>The classification based on the state of mutation IDH1/2 and the level of expression of Ki67 distinguishes subgroups biologically distinct and provides prognostic information regardless of the WHO histopathological grade. It could be more convenient for clinical application and personalized care in Astrocytomas.</td>
</tr>
<tr>
<td>Chen et al.</td>
<td>2015</td>
<td>Asian Pac J Cancer Prev</td>
<td>China</td>
<td>There is a statistically significant association between the increase in the expression of Ki67 and the worsening in the prognosis of patients with glioma. When stratified by the cutoff point, the subgroup with values lower than 10% showed better survival than the others.</td>
</tr>
<tr>
<td>Grell et al.</td>
<td>2015</td>
<td>Clinical Oncology</td>
<td>Australia</td>
<td>Ki67 &lt; 10% in the immunohistochemical examination of recurrence was associated with a lower survival time. The change in anti-Ki67 between the initial treatment and the reoperation was also associated with survival, a lower reduction than 50% showed a worse prognosis compared with more than 50% of reduction.</td>
</tr>
</tbody>
</table>

WHO = World Health Organization (WHO); IDH1/2 = Isocitrate dehydrogenase 1 and 2.

**Discussion**

The improvement of prognosis and greater effectiveness in the treatment of astrocytomas seems to depend on the knowledge and manipulation of molecular and gene pathways that regulate the growth of these aberrant tumors. The biomarkers have brought additional information in this regard in recent decades. 2, 3, 17, 18, 23, 25.

Regarding the disease progression, two classes of markers are recognized in oncology: prognostic and predictive. The former informs the disease behavior regardless of the treatment used and the latter provide information about the expected developments if certain intervention is performed 4, 24.

According to the work of Thuy et al., who performed a systematic review of molecular and genetic markers on survival of gliomas in 14678 patients, there are four main biomarkers: the methylation of 06-methylguanine methyltransferase (06-MGMT), the mutation of isocitrate dehydrogenase 1 and 2 (IDH1/2), the rate of cell proliferation (Ki67/MIB1) and the loss of heterozygosity of chromosome 10/10q (LOH 10/10q) 26.

The rate of cell proliferation suggests the degree of neoplasms quantitative growth. A variety of methods have been used to estimate this value in tumors of CNS. Out of these, the most widely used is based on the immunohistochemistry reaction with anti-Ki67 or with the anti-MIB1 3, 4, 18, 20.

The anti-Ki67 is a monoclonal IgG1 antibody which was discovered by in 1983. It recognizes a protein existing in proliferating cells and absent in quiescent cells. The antigen is present in all phases of the cell cycle, except in the G0 and the beginning of G1. The specific function of the protein Ki67 still remains unknown 4, 19, 20, 25.

This antibody can be used only in fresh tissue, since the fixing in formaldehyde significantly reduces its immunoreactivity. However, the discovery of antibody MIB1 allowed the recognition of antigen in tissues fixed in formalin and embedded with paraffin, which has improved the detection of antigen and allowed both prospective and retrospective studies to use this marker 20.

Several studies point to a substantial increase in the rate of cell proliferation, measured by the anti-Ki67, with the increase of the gliomas malignity, with statistically higher differences
among astrocytomas of high grade when compared to those of low grade. In the work of Skjulsvik et al., the values of anti-Ki67 were similar between gliomas grade I and II and between the gliomas grade III and IV, more statistically different between low-grade and high-grade gliomas.

Saha et al. showed, with the help of the Kaplan-Meier curve, that astrocytomas with anti-Ki67 greater than 14.3 had a lower average time of survival and cumulative survival. But according to Uehara et al., anti-Ki67 is not only a prognostic marker, but also predictive, because the higher the rate of proliferation, the higher the rate of recurrence in multiform glioblastomas, even when treated correctly.

A systematic review with meta-analysis, published by Wen-Jie et al., showed a statistically significant association between the expression increase of anti-Ki67 and the worsening in the prognosis of patients with glioma. Whereas, in the patients with values lower than 10%, there was an inverse effect, with an improvement in survival.

The anti-Ki67 may be a biomarker of poor prognosis also in the postoperative period of the recurrences of astrocytomas. In a study done by Gzell et al., the value of the rate of proliferation higher than 10% at the immunohistochemical examination of recurrence was associated with a lower survival time. In the same way, the change in anti-Ki67 between the initial treatment and the reoperation was also associated with survival, a lower reduction than 50% showed a worse prognosis compared with more than 50% of reduction.

In the majority of studies, the rate of cellular proliferation is used as a prognostic marker, however, there are articles that mention the importance of anti-Ki67 as a method of gliomas classification. Values above 10% seem to be a cut-off point for differentiating the astrocytomas in benign and malignant tumors.

According to Zeng et al., the classification based on the state of mutation IDH1/2 and the level of expression of Ki67 distinguishes biologically distinct subgroups and provides prognostic information regardless of the WHO’s histopathological grade. According to the author, the astrocytomas can be divided into five groups, being this classification the most convenient for clinical application and to guide the treatment of these tumors.

However, the values of anti-Ki67 are very variable, since the counting methods undergo a lot of interobservers and intraobservers variation and there is no standardization of immunohistochemical procedures among different laboratories.

Therefore, despite of the anti-Ki67 being an important clinical marker, it must be used in combination with other variables, such as imaging examinations, neurological conditions, duration of symptoms and histopathological criteria to define the diagnosis and prognosis of the disease.

**Conclusion**

High levels of Ki67 are associated to high grade astrocytomas and to a lower survival time. A value greater than 10% is a cut-off point reasonable to indicate a greater potential for malignancy and worse prognosis.

Due to the great variability among their results, this marker cannot be used alone, but in combination with other variables to define the classification, diagnosis and prognosis of the disease.

**REFERENCES**


6. Sareddy GR, Nair BC, Gonugunta VK, Zhang QG, Brenner A,


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