Article is available online at http://www.webio.hu/por/2006/12/3/0143

ARTICLE

The Clinical Value of Ki-67/MIB-1 Labeling Index in Human Astrocytomas

Anne Linn JOHANNESSEN, Sverre Helge TORP

Department of Pathology and Medical Genetics, Department of Laboratory Medicine, Children's and Women's Health, St. Olavs Hospital, Trondheim University Hospital, Norway

The current WHO classification of human astrocytomas has limitations in predicting prognosis and diagnosis, and there is a need for additional factors. Several studies have investigated the clinical value of proliferative activity in these tumors, especially the Ki-67/MIB-1 labeling index (LI). The aim of this study was to review the literature on this topic to get a survey of the current experience. All studies show increasing values of Ki-67/MIB-1 LI with increasing grade of malignancy. Most of them demonstrate that MIB-1 LI differentiates well between diffuse astrocytomas WHO grade II (AII) and anaplastic astrocytomas (AA) and between AII and glioblastomas (GM), but not between AA and GM. There is, however, considerable overlap of indices between the different malignancy groups. Further, in most studies positive correlations between MIB-1 LI and survival are found, though the proposed cut-off values vary substantially between the reports. The studies reviewed report MIB-1 LI as an important prognostic factor in human astrocytomas. Due to the great spread of values between the various tumor grades, however, MIB-1 LI cannot be used as a diagnostic factor alone but should be used in combination with established criteria of histological malignancy. It may be especially useful in cases where histology reveals a low-grade astrocytoma whereas other parameters indicate a more malignant neoplasm. Thus, it is our opinion that MIB-1 LI should be a part of the routine investigation in patients with astrocytic tumors. Until larger multicenter studies based on standardized immunohistopathological procedures have been completed, each laboratory has to establish its own practice. (Pathology Oncology Research Vol 12, No 3, 143–147)

Key words: Brain-tumors, gliomas-immunohistochemistry, Ki-67, prognosis

Introduction

Astrocytomas are the most common primary intracerebral tumors. Although low-grade astrocytomas have a tendency to grow slowly, they may progress to higher grade types as anaplastic astrocytomas or glioblastomas. Since the current WHO classification of astrocytomas has some limitations in predicting the clinical outcome and survival, there is a need for additional diagnostic and prognostic measures. In this regard, many studies have focused on the clinical value of the proliferative activity in these tumors, especially Ki-67/MIB-1 labeling index (LI).

Received: March 3, 2006; *accepted:* June 18, 2006

Correspondence: Sverre H TORP, MD, PhD, Department of Pathology and Medical Genetics, St. Olavs Hospital, Trondheim University Hospital, N-7006 Trondheim, Norway. Tel: +47 72 57 33 65, fax: + 47 72 57 64 28, e-mail: sverre.torp@ntnu.no

Ki-67 is an IgG1 class monoclonal antibody that was discovered by Gerdes et al in 1983.[3] It recognizes a core antigen present in proliferating cells and absent in quiescent cells. The antigen is expressed in all phases of the cell cycle except for G0 and the early parts of G1. However, the amount of expressed antigen in different phases of the cell cycle may vary. The precise function of the Ki-67 protein is still unclear.

Initially, it was a practical problem that the Ki-67 antibody could only be used on fresh or frozen tissue, as fixation greatly reduced the immunostaining. The discovery of MIB-1 antibody, however, that could recognize the Ki-67 antigen in formalin-fixed and paraffin-embedded tissue sections, greatly improved the value of the detection of Ki-67 antigen.[8] Both retrospective and prospective studies now can be performed to investigate the clinical value of the Ki-67 antigen as a marker of proliferation for prognostic and diagnostic purposes.

Table 1. **Survey of reports reviewed with comments**

Authors	*No. of tumors*	*Results (all units in %)*	*Comments*
Karamitopoulou[7]	24 AII 26 AA 9 GM	MIB-1 LI (mean): AII: 2.03 AA:12.8 GM: 14.57	AII vs. AA: S AII vs. GM: S AA vs. GM: NS
Khalid et al[9]	24 AII 20 AA 33 GM	MIB-1 LI (mean): AII: 1.78 AA: 13.47 GM: 15.69	Mean MIB-1 LI was significantly higher in high-grade (III-IV) compared to low-grade astrocytomas
Sallinen et al[16]	8 AII 13 AA	MIB-1 LI (median, mean): AII: 6.4, 6.26 AA: 12.35, 16.61 Cut-off point for prognostic value: 15.3	AII vs. AA: S Best sensitivity and specificity was achieved at a MIB-1 LI level of 8%. MIB-1 LI was found to have strong prognostic potential with a cut-off point at 15.3%
Wakimoto et al[20]	19 AII 25 AA 28 GM	MIB-1 LI (mean): AII: 3.8 AA: 18.4 GM: 31.6	MIB-1 LI was an independent, statistically significant prognostic factor for patients with all grades of astrocytomas
Di et al[1]	29 AII 25 AA 24 GM	MIB-1 LI (mean): AII: 1.2 AA: 9.0 GM: 12 Cut-off point for prognostic value: 8.0	AII vs. AA: S AA vs. GM: S MIB-1 LI <8.0% was associated with longer survival both at 5 and 10 years
Hsu et al[6]	16 AII 31 AA 33 GM	MIB-1 LI (mean): AII: 0.88 AA: 8.75 GM: 9.12 Cut-off point for prognostic value: 1.5	AII vs. AA: S AII vs. GM: S AA vs. GM: NS MIB-1 LI >1.5% was associated with significantly poorer prognosis
McKeever et al[11]	14 AII 15 AA 36 GM	Cut-off point for prognostic value: 2.5	MIB-1 LI ≤2.5% was associated with longer survival
Schiffer et al[17]	50 AII	Cut-off point for prognostic value: 8.0	A MIB-1 LI cut-off >8% was associated with significantly poorer survival
Eneström et al[2]	6 AII 9 AA 7 GM	MIB-1 LI (mean, median): AII: 7.6, 7.5 AA: 13.3, 14.0 GM: 24.3, 27.0 Cut-off point for prognostic value: 10.0	AII vs. AA: S AA vs. GM: S MIB-1 LI of 10% divided the astrocytomas into low- and high-grade
Hilton et al[5]	96 AII	MIB-1 LI (mean, median): 1.15, 0.49	MIB-1 LI did not correlate with survival in either the irradiated or the non-irradiated group, nor with overall survival in the series as a whole
McKeever et al[12]	50 AII	MIB-1 LI was ≤2.0 in 22 patients and >2.0 in 28 patients. Over a median follow-up of 10 years, 23% and 82% died of tumor in the first and second group, respectively	MIB-1 LI >2.0% indicated poorer prognosis

Table 1. **Survey of reports reviewed with comments**

Authors	*No. of tumors*	*Results (all units in %)*	*Comments*
Ralte et al[14]	30 AII 11 AA 15 GM	MIB-1 LI in initial tumors (mean): AII: 3.73 AA: 9.65 GM: 10.33 Cut-off point for prognostic value: 2.8	AII vs. AA: S AII vs. GM: S AA vs. GM: NS MIB-1 LI >2.8% cases had shorter interval to recurrence and poorer prognosis
Reavey-Cantwell et al[15]	32 GM	Cut-off point for prognostic value: 20.0	Multivariate analysis indicated that patients with MIB-1 LI >20% had 2.2 times the risk of death compared with patients with LI <20%
Torp 2002[18]	22 AII 10 AA 9 GM	MIB-1 (median): AII: 2.7 AA: 13.9 GM: 12.1 Cut-off point for prognostic value: 5.2	AII vs. AA: S AA vs. GM: NS MIB-1 LI >5.2% for the whole tumor group was associated with significantly poorer prognosis
Torp and Alsaker[19]	22 AII	MIB-1 (median): 2.7 Cut-off point for prognostic value: 2.7	MIB-1 LI >2.7% was associated with poorer prognosis
Neder et al[13]	10 AII 5 AA 25 GM	MIB-1 LI (mean): AII: 2.35 AA: 6.44 GM: 12.28 Cut-off point for prognostic value: 3.0	MIB-1 LI >3.0% was associated with significantly shorter survival time of 12 months vs. 45 months in MIB-1 LI <3.0% cases. There was also a significant difference in the mean MIB-1 LI between patients with grade II vs. high-grade astrocytomas (1.70% vs. 11.47%)

AII: astrocytomas grade II; AA: anaplastic astrocytomas; GM: glioblastoma; 2-y: 2-year survival, S: significant; NS: not significant

There are many reports on the clinical use of Ki-67/MIB-1 LI in astrocytomas, and its significance varies from one study to another. The aim of this study was therefore to review some of these articles to get a survey of the reported diagnostic and prognostic significance of MIB-1 LI.

Materials and Methods

Studies considered for this paper were searched for by applying Pub-Med® using the following key-words: astrocytic tumors, MIB-1, Ki-67, gliomas, and proliferation. Approximately 120 papers were found by these key-words and 16 publications[1,2,5-7,9,11-20] met our inclusion criteria that were:

(1) Studies that focused on prognostic or diagnostic role of MIB-1 LI in diffuse astrocytomas grade II (AII), anaplastic astrocytomas (AA) and glioblastomas (GM) according to the WHO classification from 2000.[10]

(2) Studies that included at least 20 patients.

(3) Studies that reported scientific results at a significance level of at least 0.05.

A list of papers reviewed is shown in *Table 1*, comprising 420 patients with AII, 190 of AA, and 305 of GM. Wilcoxon test was used to compare the groups, and P values less than 0.05 were considered to be significant.

Results

The main findings and conclusions from the 16 reviewed articles are presented in *Table 1*. All studies show an increase in MIB-1 LI with the increasing grade of malignancy. Mean values (± SD) for grade II, III and IV tumors were for the whole material 3.0 (±2.1), 11.8 (±3.4) and 15.8 (±7.4), respectively. There was a statistically significant difference between the indices of low- (grade II) and high-grade tumors (grade III and IV) ($P<0.05$), but not between grade III and IV tumors ($P>0.05$). Even so, considerable overlap of values between the different tumor groups were recorded as illustrated in *Figure 1*. Most studies also demonstrate positive correlations between MIB-1 LI and survival. Proposed cut-off values are presented, though they vary from one study to another.

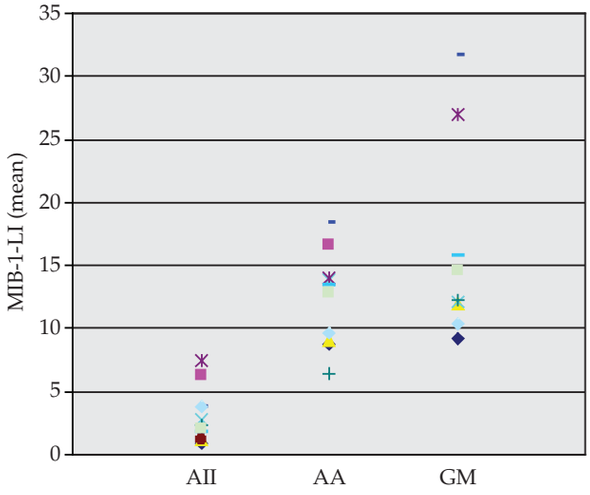

Figure 1. *Distribution of MIB-1 LIs in the different grades of astrocytomas in the reviewed studies. AII: diffuse astrocytoma WHO grade II; AA: anaplastic astrocytoma; GM: glioblastoma*

Discussion

In the treatment of malignant tumors the clinical decision-making is funded on evidence-based medicine and personal experience. Tumor grading is one of the most significant predictors for clinical outcome. Astrocytomas, the most common type of primary intracerebral tumors, are not an exemption in this respect, however, the current WHO classification of astrocytomas has limitations in predicting the clinical outcome and survival, and additional prognostic markers are welcome.

This study evaluates the clinical usefulness of proliferative activity in astrocytomas assessed by Ki-67/MIB-1 LI, based on review of 16 studies comprising a total of 915 patients. To assure a selection of papers that were comparable, we selected only studies with a reasonable number of patients, which used the WHO 2000 grading criteria of diffuse astrocytomas, and investigated either the prognostic or the diagnostic role of MIB-1 LI in these tumors.

The reports demonstrated a substantial increase in MIB-1 LI with the increasing grade of malignancy. Although the data must be pooled with care, they show an average value of MIB-1 LI in the three groups of AII, AA and GM of approximately 3, 12, and 16, respectively. This strongly suggests that increasing levels of MIB-1 LI are associated with more severe disease.

Regarding diagnostic value, most studies show statistically significant differences in MIB-1 LI between high-grade (grade III and IV) and low-grade (grade II) astrocytomas. Some studies also identify significant differences when comparing grade II with grade III and grade II with grade IV, but not when comparing the differences between grade III and IV.[6,7,9,13,14,16,18] In contrast, other reports indicate statistically significant differences in MIB-1 LI between grade III and IV tumors as well.[1,2,20]

Although most studies conclude with statistically significant differences between low- (II) and high-grade (III-IV) astrocytomas, the average level of MIB-1 LI in the different tumor groups varies considerably. This dilemma may partly be illustrated by comparing the study of Hsu et al[6] and that of Eneström et al,[2] which found mean levels of MIB-1 LI in glioblastomas of 9.12% and 24.3%, respectively. Furthermore, the reports revealed overlap of MIB-1 LIs between the different grades. Actually, values of the glioblastoma group can be as low as those for grade II tumors, indicating that MIB-1 LI cannot be used alone as a diagnostic measure.

The majority of the reviewed studies show prognostic significance of MIB-1 LI both regarding survival and recurrence, with proposed clinical cut-off values that vary greatly from one report to another, from 1.5% to 15.3%.[1,2,6,11,13-19] Thus, it is difficult to compare results between different reports and to draw clear conclusions. Nevertheless, a MIB-1 LI larger than 10% seems to be a reasonable guideline value to indicate an astrocytic tumor with increased malignant potential.

The great spread of Ki-67/MIB-1 LIs may be due to several factors, including tissue processing, immunohistochemical procedures, and interpretations of the immunostaining. Other sources of error may be tumor heterogeneity and tissue sampling.

In conclusion, this review demonstrates that MIB-1 LI serves as an important clinical marker in human astrocytomas. It should, however, be used judiciously and in combination with other variables such as radiography, clinical status, duration of symptoms, and established histopathological features of anaplasia. MIB-1 LI may be of particular importance in cases with low-grade histology when other factors indicate a more malignant neoplasm. The wide range of MIB-1 LIs in the different reports for each malignancy group makes unequivocal interpretations and comparisons of the current data difficult. There is a need for larger multicenter studies based on standardized procedures both regarding tumor grading and MIB-1 immunohistochemistry. Anyhow, it is our opinion that the determination of MIB-1 LI should constitute a part of routine investigations in patients with astrocytomas. Until further notice each laboratory has to establish its own routines and experience with this prognostic marker.

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