Labeling index in pituitary adenomas evaluated by means of MIB-1: is there a prognostic role? A critical review

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Objective: The present article presents an overview of the literature, and analyses the methods and the primary questions related to assessment of proliferation index using the Ki-67/MIB-1 labeling index in pituitary adenomas. Although atypical adenomas are characterized by their atypical morphological features by an elevated mitotic index, a Ki-67 (MIB-1) labeling index greater than 3% and extensive nuclear staining for p53, use of the proliferation index (LI) of pituitary adenomas in assessing the degree of tumor aggressiveness is a controversial topic in the literature, and there are disparate results involving many studies.

Methods: A review of literature was carried out to correlate the role of Ki-67 LI and its correlation with clinical findings, tumor size, invasiveness, recurrence, adenoma subtype, adenoma doubling time, and pituitary carcinomas is addressed.

Results: The prognosis cannot be predicted on the basis of the Ki-67 LI alone. Although there is no direct relation between Ki-67 LI and some of these variables and controversial data were found regarding some topics, our review justify the use of Ki-67 in the analysis of pituitary adenomas as an additional information for clinical decision.

Conclusion: Although assessment of proliferative may be helpful in predicting subsequent tumor recurrence or invasiveness, there are many other important and as yet unidentified factors pituitary tumors. It is clear that further research is needed to clarify these molecular mechanisms to predict those with a potentially poor clinical outcome.

Keywords: Pituitary adenomas, Labeling index, Cell growth, Invasion, MIB-1, Ki-67

Introduction

Anterior pituitary adenomas usually are histologically benign, with well defined behavior. However, in about one-third of cases, these adenomas infiltrate the surrounding tissues including the wall of the cavernous sinus. This local invasion adversely affects the surgical results and contributes to the possibility of relapse.[1–8]

It is well accepted that brain tumor growth results from the relative proportion of cells contained in three populations: (1) cycling/proliferative; (2) quiescent (GO)/static; and (3) terminally differentiated/dying.[9] Ki-67 expression is detected by the monoclonal antibody MIB-1 and is expressed as a percentage of immunopositive nuclei in the form of a Ki-67 LI. Generally, pituitary tumor with aggressively behavior has increased Ki-67 LI.

The cells in S phase (DNA duplication) were assessed in the past by means of autoradiographic analysis using [^{3}H]thymidine, and in the 1980s with bromodeoxyuridine (intravenous infusion of 5-bromodeoxyuridine, BrdU, 200 mg/m^2), to label tumor cells in the deoxyribonucleic acid (DNA) synthesis phase, S phase.[10–12] The same autoradiographic principle, a flow cytometry method was also used for the same purpose. Using flow cytometry in 21 pituitary adenomas, Nagashima *et al.* showed that the S phase fraction was less than 0.1% in nine cases, 0.1–0.5% in seven, and greater than 0.5% in five.[12] Except for two cases of Nelson's syndrome, in which it was greater than 1%, the S-phase fraction did not correlate with

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any other variables, including patient age, tumor size, or duration of signs and symptoms. The small S phase fraction of most pituitary adenomas correlates well with the clinical behavior of these tumors, which grow much more slowly than other types of brain tumors such as gliomas.[12] The higher S phase fractions may reflect aggressive and invasive growth. These methods were abandoned because of potential side effects related to teratogenicity and myelosuppression.

The cycling compartment (G1, G2, M-mitosis, and S phases) can be detected by a mouse monoclonal Ki-67 antibody; an available, rapid, safe, sensitive, and specific method for immunostaining proliferative cells.[9,13–19] The monoclonal antibody Ki-67 reacts with a human nuclear cell proliferation-associated antigen that is expressed in all active parts of the cell cycle. Cattoretti *et al.*[20] raised monoclonal antibodies, MIB 1-3, against recombinant components of the Ki-67 antigen, and showed that these antibodies were true Ki-67 equivalents, as demonstrated by immunostain of fresh specimens, along with biochemical, and molecular biological techniques. These authors used formalin-fixed, paraffin-embedded sections routinely processed for immunohistochemistry in this study, antibodies MIB-1 and MIB-3 labeled mitotic figures, while non-mitotic proliferating cells did not label under these conditions. However, when dewaxed microwave oven-processed paraffin sections of formalin-fixed tissues were used, MIB-1 and MIB-3 produced strong nuclear staining of those cells presumed to proliferate under a variety of normal and neoplastic conditions. Moreover, routine decalcification or depigmentation techniques did not alter the immunoreactivity of MIB-1 and MIB-3 with microwave-processed paraffin sections.[20,21]

The Ki-67 monoclonal antibody has been used in many types of tumors, including tumors of the central nervous system (CNS)[15,22,23] and in skull base tumors.[9,13,14,24–26]

Burger *et al.* described assessment of the proliferation indices in pituitary adenomas in their analysis of proliferating cells in tumors of the CNS.[22] However, the first article that assessed the proliferation-associated Ki-67 in fresh-frozen pituitary adenoma specimen was published by Landolt *et al.*[27] Subsequently, extensive studies have attempted to correlate the Ki-67/MIB-1 labeling index (LI) with many clinical parameters and other biological markers.

LI may be defined as the proportion of labeled cells to the total number of cells analysed in a field consisting of between 100 and 1000 cells (arbitrary definition), in the area where the density of labeled cells is the highest.[9] As a rule, the LI measured by Ki-67 shows an average rate of $1.9 \pm 1.3\%$ in pituitary adenomas.[9]

Beyond any doubt, the MIB-1 monoclonal antibody is one of the most important immunocytochemical markers for proliferation and its evaluation in routinely processed paraffin-embedded tissue specimens of pituitary adenomas.[15–17] Other factors such as the pituitary tumor transforming gene (PTTG), p16, p27, p53, vascular endothelial growth factor (VEGF), topoisomerase II-alpha, D1 cyclin, metalloproteinases, cadherin expression, cyclooxygenase, and aquaporin may also be studied, to be characterized in parallel with the MIB-1/Ki-67 LI. Together with the MIB-1/Ki-67 LI, these markers add information regarding the true biological role of cellular proliferation in a pituitary tumor.

Comparisons among the main methods that measure the LI were performed by Morimura *et al.*[28] This study showed that *in vitro* BrdU labeling can be a useful alternative to Ki-67 immunolabeling of human brain tumor specimens. Owing to collateral negative effects of BrdU, Ki-67 is the current choice for proliferation studies.[28]

The development of novel chemotherapy or gene transfer techniques is based on advances in our understanding of the genetic basis of pituitary adenoma invasiveness and proliferation. It is necessary to understand which factors may interfere with growth and its interruption, and which environmental factors might be involved in the genesis of these tumors. This is a comprehensive review of published data, serving as background for further studies.

The following topics are addressed in this review. We begin reviewing the aspects of immunocytochemical technique. Then we reviewed the relation of Ki-67 LI with clinical findings, tumor size, invasiveness, recurrence, adenoma subtype, adenoma doubling time, and pituitary carcinomas.

Aspects of the Immunocytochemical Technique

MIB-1 immunostaining is a simple and practical method that can be used in the routine histological evaluation of brain tumors and also of pituitary adenomas. Although automated analysis is faster and easier, manual cell counting is equally reliable and is applicable everywhere. A high growth fraction expressed by a high Ki-67 LI might suggest the need for careful clinical and radiological follow-up.[29–31]

Tumor cell proliferation has been assessed immunohistochemically by means of the MIB-1 antibody epitope of the Ki-67 LI antibody. The method may be used in paraffin-embedded specimens and in frozen sections. Microwave processing is necessary for paraffin sections, in order to promote deparaffination. Sections are cut at 4 μm. Antigen retrieval is obtained by boiling sections in citrate buffer. The chromogen used is a well-known immunohistochemical stain with avitin/biotin or peroxidase/anti-peroxidase (APAAP) diaminobenzidine. MIB-1 is apparent as nucleolar staining, related to fibrillar proteins in nucleoli, in the cells in the S, G1, G2, and M phase of the cell cycle. In each specimen, a total of at least 1000 tumor cell nuclei are evaluated. The count of

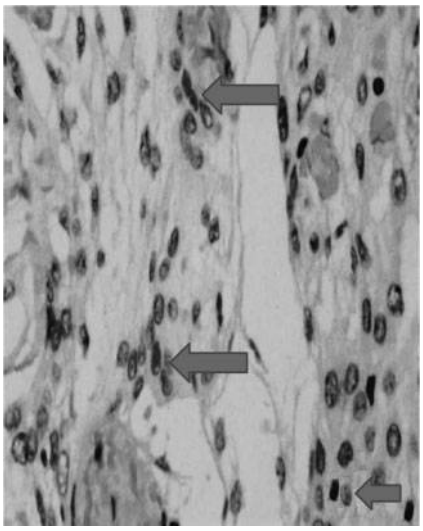

Figure 1 The histological specimen shows cells labeled immunohistochemically using the monoclonal antibody MIB-1, reaction APAAP (peroxidase anti-peroxidase), with diaminobenzidine as chromogen (dark brown nuclei), ×200. The red arrows show high density areas of labeled cells. Specimen obtained from a non-functioning pituitary adenoma, with suprasellar extension and invasion of the cavernous sinus. The estimated LI was 3% in the region of the highest density of labeled cells.

labeled cells obviously depends on interobserver variability and sampling error.[15,16,20,30,31,32] Because MIB-1 depends on tissue processing, staining method, and method of assessment, variability of results may be expected; however, results typically fall within a limited statistical spectrum. The ideal cut point for distinguishing the group of normal slow growth from faster growth is between 1.5 and 3%.[33,34] Figure 1 is placed in order to illustrate the concept and practical application of the technique.

Mastronardi *et al.*[35] used the classical method[9,20,21] using avidin–biotin–peroxidase. Ten fields were selected in regions with the highest concentrations of MIB-1-positive nuclei and were examined high power (×400). Each field corresponded to a total number of cells ranging from 700 to 1000. Areas of necrosis, normal adenohypophysial cells, and endothelial cells were excluded from the evaluation. Considering 1000 cells with 'manual' counting, the Ki-67 LI has been defined as the percentage of MIB-1 positive cells (dense brown precipitate restricted to the nuclei). In our opinion, this is the standard method to be followed.

Is There Any Correlation between the Clinical Course of Pituitary Adenoma Patients and the Ki-67/MIB-1 LI?

Age

As described by the majority of authors in the reviewed literature,[36–39] there is an inverse correlation between Ki-67 LI and age for non-functioning adenomas. However, this finding is not described in other studies.[40–42]

Gender

Several studies showed no correlation between Ki-67/MIB-1 LI and gender.[41,42] However, Delgrande *et al.*[43] demonstrated statistically higher LI in males compared to females with large prolactinomas. Paek *et al.*[39] and Qian *et al.*[44] confirmed the studies of Delgrande *et al.*[44] Paek *et al.* found similar results in microadenomas.[39]

A predominance of higher LI in females was found by Wolfsberger *et al.* in 2004 regarding non-functioning pituitary adenomas.[45] Schaller[47] studied growth hormone (GH) secreting adenomas from 18 patients (10 males) with acromegaly who met strict immunohistochemical and electron microscopic diagnostic criteria and who underwent surgical resection of their tumors. The men and women were equivalent in age at surgery.[35] Men demonstrated higher insulin-like growth factor-1 (IGF-1) and lower GH levels pre- and post-operatively, whereas the percentage reduction in IGF-1 was more pronounced in men when compared to women (58% versus 27%). The overall outcome was better in women than in men.[47] The mitosis and MIB-1 LIs were increased in men compared to women. Finally, this article concluded that clinical course and tumor biology of GH-releasing pituitary adenomas appeared to differ between women and men. Therefore, the correlation between LI and gender is unclear for pituitary adenomas.

Signs and symptoms

Ki-67 LI results have been shown to correlate with signs and symptoms related to the pituitary adenomas. According to Shaller, shorter pre-operative duration of symptoms, larger and more invasive tumors, and a worse clinical outcome were associated with higher Ki67 LI.[47] Paek *et al.* determined that visual defects correlated with higher Ki-67 LI in patients with macroadenomas.[39]

Suzuki *et al.* studied Ki-67 LI in 52 incidentally detected pituitary adenomas found lower rates of LI in tumors of non-symptomatic patients than in symptomatic patients.[42] Finally, Matha *et al.* found an association between Ki-67 LI and unilateral temporal hemianopsia in patients with non-functioning adenomas.[46] These studies suggest that the Li does correlate with tumor aggression as based on signs of mass effects and symptoms.

Pre-operative medical therapy

The Ki-67 LI is lower in octreotide-treated GH and bromocriptine-treated prolactin adenomas, suggesting that these drugs probably inhibit growth in pituitary adenomas.[37,48] Other studies have not demonstrated a statistical difference in Ki-67 LI between pre-operative drug-treated patients and untreated patients.[46,49]

Surgical treatment

There are limited data in this regard. Losa *et al.*[50] reported that a higher LI was demonstrated in

Cushing's adenomas patients not cured by surgery compared with tumors from patients in surgical remission, though better correlations occurred with maximal tumor diameter and basal ACTH levels.

Radiation therapy

Radiation therapy has an important role in the therapy of pituitary adenoma, but it is usually indicated as adjuvant therapy, and case selection is a controversial topic in pituitary tumor therapy. This controversy includes a debate concerning the choice of conventional fractionated therapy versus stereotactic radiation therapy.[51,52]

In order to elucidate histological changes in the pituitary gland and adenomas following radiotherapy, two irradiated pituitary glands and seven irradiated non-functioning adenomas were studied by Nishioka *et al.*, and it was observed that in pituitary adenomas, the MIB-1 LI remained unchanged after radiation.[53] The histological changes were more intense in adenomas following gamma knife radiotherapy than those following conventional radiotherapy alone, though the true impact of stereotactic radiotherapy versus conventional radiotherapy on the labeling index in pituitary adenomas is largely unknown.

Ki-67 Expression/MIB-1 and Tumor Size

Because of sellar confinement of pituitary adenomas, growth velocity may be reduced or inhibited in a subset of macroadenomas. Microadenomas have no restriction to growth in the sellar space and initially may have a high growth velocity, reaching a higher LI index than noted in many macroadenomas. This idea is reinforced by the study by Yonezawa *et al.*[38] As described by Turner *et al.*,[54] Ki-67 LI share significantly higher in macroprolactionomas than in microprolactinomas. This finding was not corroborated by others.[35,43]

In contrast, ACTH secretion correlates with the size of the corticotroph tumor and a high rate of MIB-1 labeling,[50] perhaps because the functional adenomas have their growth influenced by many other factors, and contact inhibition may be not so important. It suggests that tumor subtype is important when considering Ki-67 staining and tumor size.

Ki-67 Expression/MIB-1 LI and Invasiveness. Is There a Valid Correlation?

A tendency for parasellar expansion of many anterior pituitary adenomas has well been described in the literature.[55–58] Jefferson identified a group of pituitary tumors classified as 'invasive' adenomas, in which extrasellar and parasellar spread might occur.[56] An invasive pituitary adenoma was defined as a tumor that extended beyond its capsule or involved contiguous structures. The incidence of invasiveness among these tumors varies among different anterior pituitary adenoma subtypes[56,59] and also in relation to the criteria used for assessment. Table 1 summarizes the main studies correlating

Table 1 Literature review of the correlation between Ki-67/MIB-1 LI and Invasiveness in pituitary adenomas

Reference	*n*	GH	PRL	ACTH	TSH	NF	FSH and/or LH	Plurihormonal	Association with tumor invasiveness
64	32					32			No correlation
83	25		25						↑ Increased ↑ in invasive*
46	85					85			Increased in invasive
95	213					213			No correlation
65	35	2	7	1		1	5	19	↑ Increased in invasive case ↑ **
39	44	8	8			28			Negative correlation
26	1					1			Increased in invasive case
81	65	21	6	6	2	2	28		↑ Increased ↑ in invasive
66	159	43	19	16		42		39	↑ Increased in invasive**
45	260 (MIB-1)	34	64	36	4	67	39	16	↑ Increased ↑ in invasive**
68	26 (Ki-67)	8	4	4		9		1	↑ Increased ↑ in invasive**
34	23					23			No correlation
37	132	42	15	6	1	68			↑ Increased ↑ in invasive***
82	69	7	9	4	1	37	5	6	↑ ?Increased in invasive**
35	121	24	26	12	3	48	1	7	↑ Increased in invasive**
71	94	18	20	5		41		10	No correlation
62	15	15							↑ Increased in invasive*
54	160	46	29	18		67			Negative correlation
41	103	21	24	10		41		7	↑ Increased ↑ in invasive**
69	123	34	15	6		68			↑ Increased ↑ in invasive**
70	48								↑ Increased in invasive*
38	85	11	17			57			↑ Increased in invasive
40	45	9	8	1		27			No correlation
43	96		96						No Correlation
4	31	4	10	3		12	2		↑ Increased ↑ in invasive**
30	127	23	8	7	3	73		6	↑ Increased in invasive*
49	65	10	20	7	3	24			No correlation

Note: *n* = no. of cases; GH = growth hormone adenoma; PRL = prolactin adenoma; ACTH = adenocorticotropin hormone adenoma; TSH = thyroid simulating hormone adenoma; FSH = follicle stimulating hormone adenoma; LH = luteinizing hormone adenoma; plurihormonal = adenoma with two or more hormones; NF = non-functioning adenoma; ↑ = higher; *$P < 0.005$; **$P < 0.05$; ***not statistically significant.

invasiveness and proliferative index assessed by MIB-1/Ki-67 LI.

The most accepted criterion to classify invasiveness is radiological, by means of coronal sections of the MRI as suggested by Knosp *et al.*[6] The grades 0–3 are distinguished from each other by a medial tangent, the intercarotid line-through the cross-sectional centers, and a lateral tangent based on the intra- and supracavernous internal carotid arteries.[6] Grade 0 is the tumor which does not invade the cavernous sinus, and Grade 4 corresponds to total encasement of the intracavernous carotid artery. In a series of 25 surgically-treated invasive pituitary adenomas, Knosp *et al.*[6] used this classification and found surgically proven invasion of the cavernous sinus space present in all Grades 3 and 4 cases and in all but one of the Grade 2 cases; no invasion was present in Grades 0 and 1 cases. The critical area where invasion of the cavernous sinus space becomes very likely and can be proven surgically is located between the intercarotid line and the lateral tangent, which is represented as Grade 2. In this article, Knosp *et al.*[6] also measured tumor growth rates, using the monoclonal antibody Ki-67, and showed a statistically higher proliferation rate ($P < 0.001$) in adenomas with surgically observed invasion into the cavernous sinus space, as compared with non-invasive adenomas.

In macroadenomas of the pituitary gland, invasion of parasellar spaces may occur in 6–10% of cases.[60] With high-resolution high-field (3 T) MRI, the sellar region may be evaluated more accurately as compared to lower field strengths.[60]

Many authors have shown a correlation of Ki-67/MIB-1 LI with tumor growth velocity[5,34,37,61] and tumor invasiveness into skull base anatomically adjacent structures.[4,26,30,36,41,61–69] Thapar *et al.*[30] established a Ki-67 LI threshold of 3% for distinguishing between non-invasive and invasive adenomas, with specificity and sensitivity of 97 and 73%, respectively. Mizoue *et al.* suggested a cut point of 1% for non-invasive versus invasive tumors. This series included eight adenomas with rapid growth out of a total of 48 non-invasive tumors.[70]

Yonezawa *et al.*[38] found no difference in Ki-67 Li between invasive and non-invasive functioning microadenomas. In a study of 103 pituitary adenomas, Mastronardi *et al.*[35] concluded that the cut points were 3.5% for invasive adenomas and 5% for cavernous sinus involvement, much higher than proposed by Thapar *et al.*[30] The mean cut point, however, could not be used in all series, because, in some series, the LIs in invasive tumors were above 3 or 3.5%.[69] Zhao *et al.* found a mean LI of 0.75% for non-invasive adenomas compared to 2.2% in invasive tumors.[69]

Mastronardi *et al.*[41] studied 24 microadenomas, 27 intrasellar macroadenomas, 34 intra-suprasellar macroadenomas, and 36 intra-supra-parasellar macroadenomas. In their analysis, there were 76 non-infiltrating adenomas and 45 infiltrating adenomas. The wall of the cavernous sinus (CS) was infiltrated in 18 cases. Forty-eight adenomas were non-functioning and 73 were functioning.[41] The LIs were $3.73 \pm 5.13\%$ in infiltrating and $2.03 \pm 2.41\%$ in non-infiltrating adenomas ($P = 0.02$), and $5.61 \pm 7.19\%$ in CS-infiltrating versus $2.09 \pm 2.37\%$ in CS-non-infiltrating adenomas ($P = 0.0005$). This study demonstrated a higher LI in the more aggressive, infiltrating tumors.

Lath *et al.*[71] demonstrated that the LI correlated with clinical and radiological evidence of invasiveness. These authors demonstrated that the mean Ki-67 LI for all pituitary adenomas was 0.84% (range: 0–17.45%) and that voluminous invasive macroadenomas (1.44%) had a higher Ki-67 LI as compared to microadenomas (0.36%). The authors concluded that the difference in the Ki-67 LI between invasive and non-invasive adenomas was not statistically significant; hence, for their series of 94 pituitary adenomas, the Ki-67 LI was not a reliable indicator of invasiveness in pituitary adenomas.[71]

The topic is so controversial that Pizarro *et al.*[66], in a well performed study involving microadenomas, found significantly higher Ki-67 LI in invasive than non- invasive tumors. However, a well-defined LI cut point was not established due to overlap in the LI between the two groups. Paek *et al.*[39], in their study of pituitary macroadenomas, did not detect a significant difference in LI between invasive and non-invasive, as well as between non-functioning and functioning tumors.[39] Mahta *et al.*[72] studied 85 non-functioning pituitary adenomas and did not find a correlation between Ki-67 LI with either invasiveness or recurrence.

In the most recent World Health Organization Classification of Tumors of Endocrine Organs (2007), Ki-67 LI higher than 3% defines a pituitary adenoma as atypical, but based on the literature addressed above, this cutoff of 3% is controversial and, besides, even for some authors,[39,72] there is no relation between invasiveness and Ki-67.

Ki-67 Expression/MIB-1 and Recurrence: Important for Prognosis?

Because Ki-67 LI correlates with tumor proliferation, it has been suggested that Ki-67 may correlate with risk of recurrence. Abe *et al.*[40] demonstrated a recurrence rate of 50% in tumors with Ki-67 LI higher than 1.5% and a recurrence rate of 16% in tumors with LI <1%. Turner *et al.*, in non-functioning adenomas, demonstrated no correlation between Ki-67 LI and recurrence, concluding that this marker has no utility as a predictor of tumor behavior.[54] Nakabayashi *et al.*[72] emphasized that subtotal surgical resection is an important factor contributing to recurrence. In this study, the Ki-67 LI and analysis

of cyclin A rates were evaluated and found to be predictive of shorter progression-free survival.[72]

In recurring non-functioning pituitary adenomas (NFPAs), the initial MIB LI after surgery has been shown to be statistically higher than in tumors with no regrowth.[5] Though this topic is controversial, Honegger *et al.*[34] showed a significant correlation between MIB-1 LI and velocity of regrowth, which was confirmed by Tanaka *et al.*[36] No statistical difference regarding NFPAs has been observed by other authors.[37,50,73]

Some studies suggest that the regrowth potential in the absence of postoperative radiotherapy or radiosurgery may reach a rate between 38 and 95%; however, lack of consistent follow-up methodology may create a bias in the analysis.[74–78] Recurrence may be present in the 3–6 months following surgery, as detected mainly by T1 MRI in the coronal section images, with gadolinium.

However, it is important to keep in mind that some studies showed that residual tumors may remain unchanged or have minimal growth in almost 48% for a long period of time.[75,76]

In another study, Ki-67/MIB-1 was a better predictor than PTTG as a marker of recurrence, particularly with follow-up greater than 1 year.[79] The cut point of 2.9% for Ki67 for pituitary adenomas showed a higher incidence of recurrence.[79] In a series of 176 pituitary tumors, Scheithauer *et al.*[48] found no correlation of Ki-67 LI with recurrence based on follow-up of 78 patients, of whom only seven had indices higher than 3%.

The most recent World Health Organization Tumor Classification of Tumors of Endocrine Organs defines an atypical pituitary adenoma as a tumor with Ki-67 LI higher than 3%. The controversy of this definition is that these tumors are uncommon and that there is aggressive behavior in tumors with Ki-67 LI lower than 3%.

Therefore, the literature suggests that use of Ki-67 LI alone has limited prognostic ability to predict recurrence accurately, and the association with other biological markers may be more promising. The main studies in literature are summarized in Table 2.

MIB-1 LI and pituitary adenoma doubling time

Several authors have found an inverse correlation between Ki-67 LI and tumor volume doubling time which was statistically significant.[5,34,36,70] These data support a role for Ki-67 LI to predict tumor aggression.

MIB-1 LI and pituitary adenoma subtypes

It has been demonstrated that Ki-67 staining was higher in functioning tumors than in non-functioning tumors.[6,24,30,37,41,42,48,68] Some articles,[40,69,80–82] such as those published by Paek *et al.*, showed no correlation between Ki-67 LI and type of pituitary adenomas.[39] Table 3 summarizes the main studies correlating MIB-1/Ki-67 LI and pituitary adenoma subtypes.

Prolactin (PRL) secreting tumors

Asano *et al.*[1] evaluated the MIB-1 index in 63 surgically removed pituitary adenomas; values ranged from 0 to 6.5%, with lower values in growth hormone secreting tumors and high values in prolactin secreting tumors. Pre-operative treatment with bromocriptine had no effect on the LI values.[1] Mastronardi *et al.*[41] also found that growth hormone secreting adenomas had a low Ki-67 LI, whereas prolactin secreting tumors had a mean index above the mean for the cohort as a whole. Delgrande *et al.*[43] demonstrated that males harboring large macroprolactinomas have a higher LI as compared with females with PRL adenomas. Others authors reported similar results.[39,44]

Wierinckx *et al.*[83] showed that markers of proliferation could not differentiate invasive from non-invasive PRL secreting adenomas. This study

Table 2 Correlation between MIB-1/Ki-67 and recurrence of pituitary adenomas

Reference	*n*	GH	PRL	ACTH	TSH	NF	FSH and/or LH	Plurihormonal	Correlation with tumor recurrence
46	85					85			No correlation
87	32						32		No correlation
83	25		25						↑ No correlation
79	45	8	14	6		6	11		> in recurrent tumors*
31	176	20	35	18	10	50	20		No correlation
39	44	8	8			28			> recurrent tumors*
66	159	43	19	16		42		39	No correlation
34	23					23			> recurrent tumors
36	40					40			> recurrent tumors
73	51								No correlation
37	132	42	15	6	1	68			No Correlation
72	48								< progression free interval**
50	101					101			No Correlation
94	67					67			No Correlation
5	33					33			Increased velocity of growth in recurrence
49	65	10	20	7	3	24			Increased in recurrence**

Note: *n* = total; GH = growth hormone adenoma; PRL = prolactin adenoma; ACTH = adenocorticotropin hormone adenoma; TSH = thyroid simulating hormone adenoma; FSH = follicle stimulating hormone adenoma; LH = luteinizing hormone adenoma; Pluri = adenoma with two or more hormones; NF = non-functioning adenoma; > = higher; < = lower; *$P < 0.05$; **$P < 0.005$.

evaluated mitotic activity, Ki-67 LI, and p53 labeling, and demonstrated that mitotic index and Ki67 LI were elevated in five of 25 invasive tumors.

These studies suggest that LI as measured by Ki-67 may be, in part, dependent on the type of pituitary adenoma.

ACTH secreting tumors

Several authors have found a high Ki-67/MIB-1 in ACTH secreting tumors, with values similar to recurrent adenomas and non functioning adenomas.[24,30,35,49] Katznelson *et al.*[84] reported higher Ki-67 LI in macroadenomas producing ACTH (44% of macroadenomas with high LI) than microadenomas (only 18% of microadenomas with higher LI). Losa *et al.*[61] also reported that a high LI was demonstrated in Cushing's adenoma patients not in remission in the post-operative period when compared with patients in remission. Significant correlations were present with maximal tumor diameter and basal ACTH levels,[61] with results similar to those of Katznelson *et al.*[84]

Several authors have reported a higher Ki-67 LI in ACTH secreting tumors than other functioning secreting tumors even when ACTH microadenomas are included.[35,49,85] Some 'silent' corticotroph tumors may have the potential for ACTH secretion leading to hypercortisolemia at a later stage in the disease but Ki-67 does not predict this behavior.

GH secreting tumors

Shaller,[47] studying 18 GH secreting tumors by means of MIB-1 and mitosis index, suggested that therapy for GH-releasing adenomas should be more aggressive in men than in women. The gender-related differences in GH-releasing pituitary adenomas appear to have a basis in different biologic behavior, suggesting that the LI and aggressive invasion might be higher in men.[47]

GHRH mRNA was correlated with high rates of Ki-67 LI demonstrating that the aggressiveness of tumors was related to GHRH-mRNA.[86]

Gonadotrophin secreting tumors

There are limited data available for these tumors. Dubois *et al.*[87] found no correlation between Ki-67 LI and residual tumor, as well as ki-67 LI and recurrence in 32 gonadotropic pituitary adenomas. They did find a correlation of recurrence with age and antero posterior diameter.[87]

Silent and null cells adenomas

Clinically non-functioning pituitary adenomas are classified by Asa and Kovacs in two groups. The first group is related to those tumors which have hormone immunoreactivity and ultrastructural features of known adenohypophyseal cell types. The former involves the silent somatotroph adenomas, silent corticotroph adenomas, and silent gonadotroph adenomas. The second group includes silent type III adenomas, null cell adenomas, and oncocytomas. Silent corticotroph adenomas are rare tumors defined as pituitary tumors with ACTH immunoreactivity, but without clinical evidence of Cushing's disease.

The growth characteristics of clinically silent pituitary adenomas are poorly understood. Therefore, the radiologically measured growth of

Table 3 Correlation between KI-67/MIB-1 LI and pituitary adenoma subtype

Reference	*n*	GH	PRL	ACTH	TSH	NF	FSH and/or LH	Pluri	Association with tumor subtype or functional status
31	176	20	35	18	10	50	20		> in HS than NF*
42	95+9 silent +22 gonadotrophic adenomas					43S 43N S	22		> in NF than incidentalomas**
80	38	6	9					23	No statistical correlation
39	44	8	8			28			No statistical correlation
81	65	21	5	3	3	34			No statistical correlation
66	159	43	19	16		42		39	No statistical correlation
45	260	34	64	36	4	67	39		> in HS than NF**
37	132	42	15	6	1	68			> in HS than NF*
82	69	7	9	4	1	37	5	6	No statistical correlation
35	121	24	26	12	3	48	1	7	No statistical correlation
61	51			51–36 macro- and 15 microadenomas					> Ki-67 LI in macroadenomas than microadenomas**
69	123	34	15	6		68			No statistical correlation
41	103	21	24	10		41		7	> in ACTH than other HS tumor**
84	33			33–16 macro- and 17 microadenomas					> Ki-67 LI in macroadenomas than microadenomas
40	45	9	8	1		27			No statistical correlation
30	127	23	8	7	3	73		6	> in HS than NF**
39	65	10	20	7	3	24			> in HS than NF**

Note: *n* = total; GH = growth hormone adenoma; PRL = prolactin adenoma; ACTH = adenocorticotropin hormone adenoma; TSH = thyroid simulating hormone adenoma; FSH = follicle stimulating hormone adenoma; LH = luteinizing hormone adenoma; Pluri = adenoma with two or more hormones; NF=non-functioning adenoma; HS = hormone secreting tumors, S = symptomatic; NS = non-symptomatic, LI = labeling index; > = higher; $^{**}P < 0.05$; *statistically not significant.

inactive pituitary adenomas may be analysed and compared with adenoma classification and immunostaining for proliferation markers.[64] In a series of 32 patients with non-functioning pituitary adenomas who underwent 45 operations, Saeger *et al.*[64] analysed the correlation between invasiveness by means of MRI and immunostained characteristics (PCNA, MIB-1, P53 and IGF-1), and concluded that statistically significant differences were present for growth rate and PCNA expression. P53 immunostained positive in invasive adenomas only.[64] There were no correlations with clinical growth rate, but p53 expression correlated significantly to numbers of MiB-1-positive nuclei and PCNA-positive nuclei. The mean LI for MiB-1 was 0.12 in adenomas growing less than 1.5 mm per year and 0.34 in adenomas growing more than 1.5 mm per year. For non-invasive adenomas, the MiB-1 LI was 0.03, for invasive adenomas, it was 0.126, and for strongly invasive adenomas, it was 0.212. The MiB-1 LI was lower in null cell adenomas than in FSH/LH adenomas. Otherwise, all these data for MIB-1 showed no statistically significant differences ($P < 0.05$). This study found that the PCNA LI in adenomas growing less than 1.5 mm per year was 0.51 in contrast to LI of 1.12 for those growing more than 1.5 mm. In non-invasive adenomas, the PCNA LI was 0.796, in invasive adenomas, it was 0.655 and in diffusely invasive lesions, it was 1.011. Finally, these authors recommend the use of PCNA if correlations with progression of tumor growth are desired.[64]

Oncocytomas

In one study, there was no correlation between oncocytic transformation and Ki-67/MIB-1 LI.[81] Because of the rarity of this transformation, further studies are necessary to achieve a definitive conclusion

Non-functioning adenomas

Pituitary adenomas without clinically active hypersecretion are summarized under the term non-functioning pituitary adenoma.

Complete surgical removal of non-functioning adenomas has been reported in up to 16–79% of cases.[34,50,74–78,88–95] This complicates the evaluation of the influence of MIB-1 on recurrence, because depending on grade of resection, the remnant tumor volume may have a greater apparent regrowth potential.

Yonezawa *et al.*[38] showed higher Ki-67 LI in non-functioning microadenomas compared to expansive and invasive adenomas, regardless of function. Turner *et al.*[54,94] have reported no correlation between Ki-67 LI and recurrence in non functioning adenomas.

Table 4 The correlation between biological markers and Ki-67/MIB-1 in pituitary adenomas

Reference	*n*	Marker	Conclusion
106	73	Metaloproteinase MMP-9	Increased in invasion and Ki-67
64	32 (non-functioning adenomas)	P53	Correlation with PCNA and MIB-1*
83	25	PTTG P53	Indirect correlation with invasiveness and recurrence
95	213	E-cadherin MMP-9 P53 Ptd-FGFR4	No correlation in cavernous sinus pituitary adenoma invasion
42	52 (incidentalomas)	D-Topoisomerase	No correlation
79	45	PTTG	Correlation with Ki-67
80	38	c-erb2	Variable positivity for Ki-67
105	54	Metaloproteinase MMP-2	No correlation with Ki-67
104	117	c-erb B2	No correlation with Ki-67
65	35	MVD (F8) VEGF c-Myc bcl 2 MMP-9	Indirect correlation in invasive mainly MVD and VEGF MMP-9* No correlation c-myc,bcl-2
45	260	D topoisomerase II Alpha	Increased in higher rates of MIB-1**
44	39 (13 invasive, and 26 non-invasive)	E-cadherin/beta cadherin	Increased in tumors with high rates of Ki-67
82	69	P53/Mdm2	Increased in invasive tumors and Ki-67 increased in invasive tumors
72	48	A ciclin/p27	No correlation
54	160	Bcl 2, MVD	Increased in invasive tumours, and Ki-67 also increased
94	67	Cyclin A, B, D, and E	Cyclin A, B, D and E higher in macroadenomas than microadenomas. In NF adenomas higher cyclin D and Ki-67
62	25(secreting GH)	VEGF	Increased and Ki-67 increased in invasive tumors
69	123	P27	No correlation
86	91 (secreting GH)	GHRAmRNA	Linearly correlation with Ki-67 LI
30	12 (invasive)	P53	P53 and Ki-67 higher in invasive adenomas

Note: MMP = metaloproteinases; PTTG = pituitary transformer tumor gene; Mdm = murine double minute; PCNA = proliferative cell nuclear antigen minute; VEGF = vascular endothelial growth factor; MVD = microvessel density; NF = non-functioning; GHRH-mRNA = gene releasing hormone receptor; ptd-FGFR4 = pituitary tumor-derived fibroblast growth fraction receptor 4; *statistic significance;**statistically not significant.

Suzuki *et al.*[42] demonstrated that Ki-67 LI was higher in non-functioning tumor than secreting adenomas in a group of small tumors.

It is necessary to mention that most 'non-functioning adenomas' are in fact gonadotroph adenomas.

Incidentalomas

Some authors found lower values of LI values in incidentally discovered pituitary masses, mostly from autopsy studies, than pituitary adenomas in symptomatic patients. In a study of eight incidental pituitary microadenomas identified from 120 pituitary glands procured from MIB-1, staining for the Ki-67 LI antigen showed absolutely no expression.[96] The low proliferation indices in these tumors may reflect the small tumor size.[42,96]

The correlation of others biomarkers (PTTG, securin, H-ras, c-Myc, c-erb, P53, nm23, Rb genes, bcl-2, cyclin, p27, cadherins and catenins, and metalloproteinases) with Ki-67 LI in pituitary adenomas can give us more information regarding biological behavior of these tumors,[44,49,54,68,72,75,79,82,89,97,98,100,101–106] but is not the purpose of this article discuss these topics. Table 4 summarizes the main studies concerning Ki67/MIB-1 LI and their correlations with other biological markers for pituitary adenomas.

Pituitary carcinoma

These tumors are defined by the presence of brain, subarachnoid, or systemic metastasis. Pituitary carcinomas are rare and, although there is a higher Ki-67 LI (range: 7.8–11.91%), a progression of an adenoma-to-carcinoma sequence is not totally established: *de novo* pituitary tumorigenesis or a separate clonal expansion from the original tumor should be considered.

Conclusions

Currently, the main method for evaluating proliferation index in pituitary adenomas is immunohystochemical analysis by means of the monoclonal antibody MIB-1 to the Ki-67 LI antigen found in the protein of nucleoli of proliferating cells. Ki-67 can be measured in archived paraffin tissues or in frozen sections. There are also many other bio-molecular markers and genetic factors that may be important in understanding proliferation and invasiveness of pituitary adenomas. Further studies are necessary to determine the value of these markers in predicting tumor aggressiveness, and whether individual markers may have greater specificity with pituitary tumor subtypes.

Tumor size, invasion, and the incomplete tumor resection are important risk factors for recurrence or progression of pituitary adenomas.[28] Patient with progressive residual tumors showed invasiveness significantly more frequent than stable tumors.[107] The next investigations should find a Ki-67 LI threshold for post-surgical tumor resection to define more precisely information regarding tumor progression. There is no evidence that an adenoma with higher Ki-67 LI will progress to carcinoma.

This review justifies the use of Ki-67 LI in the analysis of pituitary adenomas as a piece of additional information for clinical decision making because a high Ki-67 LI proliferative index in a pituitary adenoma might indicate a more aggressive behavior. We believe that the Ki-67/MIB-1 LI represents an additional piece of information that is helpful for clinical decision making. An adenoma with a high Ki-67 LI has an increased risk of early recurrence and invasiveness, and may need closer follow-up and aggressive adjuvant therapy. However, the overlap of Ki-67/MIB-1 LI, particularly in those adenomas with moderate growth velocity, suggests that the prognosis cannot be predicted on the basis of the Ki-67/MIB-1 LI alone.

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