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Proliferation rate of intracranial meningiomas as defined by the monoclonal antibody MIB-1

Correlation with peritumoural oedema and other clinicoradiological and histological characteristics

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Abstract Paraffin-embedded surgical specimens from 55 meningiomas were immunostained after microwave processing using the streptavidin/peroxidase method and the monoclonal antibody (moAb) MIB-1 to the Ki-67 antigen. The authors assessed proliferative labelling index (LI) from a series of surgically removed meningiomas using immunohistochemical methods and MIB-1, and they correlated this index with clinical, radiological, and histological factors. No relationship was found between LI, sex, age, resection and histological grades, or volume. Symptoms, location, and peritumoural oedema did have a significant relationship to the MIB-1 LI. The symptomatic patients, i.e. those with tumours at the base of the skull and with GR3 peritumoural oedema (grade 3), had a greater chance of higher MIB-1 LI. It was proven that the increase of one unit in peritumoural oedema classification gave an increased risk of 3.312 and an LI greater than 3%. The authors also discuss the different methods of evaluating LIs in meningiomas, based on the available literature.

Keywords Brain tumour · Immunohistochemistry · Meningiomas · MIB-1

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Introduction

Tumours derived from meningothelial cells are among the most frequent neoplasms in the central nervous system. Fortunately, the majority of cases are clinically benign, and some are even found to be asymptomatic. Meningiomas are generally well confined, slow-growing lesions that are responsive to complete surgical removal, and they account for 13–19% of all brain tumours treatable by surgery [59]. The frequency of intracranial meningiomas in women is approximately twice that found in men. Their size can increase during pregnancy and they are described as being associated with breast cancer [8].

Aggressive growth has been noted in these tumours. Recently, there have been attempts to define a subgroup of malignant meningiomas histopathologically. One of the major problems encountered is in the discrepancies between the histological morphology and behaviour of these tumours. The proliferative potential of meningiomas is variable; some of them keep their size for long periods of time, whereas others grow much more rapidly. Cell cycle studies are also of major importance in human neuro-oncology; accurate diagnosis of the proliferative potential of human brain tumours may be useful when assessing and deciding upon treatment in specific cases [1, 6, 25].

For the past two decades, autoradiographic studies of tumours exposed to trytriated thymidine in vivo or in vitro [23, 24] together with immunohistochemical localisation of bromodeoxyuridine (BUdR) [60], flow cytometry assessment of DNA particles in tumour cells [5, 30], evaluation of the numbers of silver-stained nucleoli in the organiser region-associated proteins (AgNOR) [22, 43], and immunohistochemical evaluation of the expression of proliferating cell nuclear antigen (PCNA) [14, 27, 34, 50, 55, 62, 75] have all been used to evaluate the proliferative index in malignant tumours.

The Ki-67 monoclonal antibody (moAb) was recently introduced for use in frozen sections [12, 15, 16, 17, 18,

19, 53, 57, 58, 64, 70, 72, 73, 76], and a modification of the Ki-67 protein gave rise to the MIB-1 moAb, which proved suitable for use in archival paraffin sections [2, 3, 4, 28, 35, 36, 40, 49, 51, 52]. This antibody recognises a nuclear antigen which appears in all phases of the cell cycle except the G0 phase [16, 17] This nuclear antigen is mainly found in the nuclear cortex and in dense fibrillary components [72, 73]. It has been documented that Ki-67 and MIB-1 show an identical immunostaining pattern [7, 13, 46, 61].

The purpose of this study was to assess the proliferative index in a series of surgically removed meningiomas using immunohistochemical methods with MIB-1 moAb and to correlate this index with clinical, radiological, and histological factors.

Patients and methods

Fifty-five intercranial meningiomas surgically removed by the main author from September 1993 to September 1997 were included in the study. Preoperative computed tomography (CT) and magnetic resonance imaging (MRI) were made available to all patients except those with multiple meningiomas and neurofibromatosis stigmata. Clinical, surgical, pathological, and neuroimaging reports were reviewed in all cases.

Preoperative clinical condition was assessed according to the modified scale of Kallio et al. [33]: class 1 patients with no neurological symptoms or those with neurological symptoms but no neurological deficits, class 2 with slight neurological deficits, and class 3 requiring acute hospital care due to impaired consciousness and/or permanent hospitalisation during the postoperative stage.

The extent of surgical removal was graded in accordance with Simpson [66] grades: I (complete resection), II (complete coagulation of the dural insertion), III (incomplete resection leaving a small section of tumour), IV (incomplete resection leaving a moderate section of tumour), and V (minimal or no resection). For histological examination purposes, samples were set in formalin and embedded in paraffin, and slides were stained with haematoxylin and eosin (H&E) and trichrome of Masson where indicated. Meningiomas were classified according to the WHO classification [37], whether benign, atypical, or malignant.

Preoperative CT and MR images

Preoperative CT and MR images determine the location and size of the tumour together with the extent of the peritumoural oedema. Twenty tumours were located in the convexity of the brain. Twenty-eight were in the parasagittal region and falx, two in the sphenoid ridge and cavernous sinus, three in the olfactory groove, one in the foramen magnum, and one adhering to the tentorium. Forty-nine tumours were supratentorial, with no attachment to the skull base (group A), and six (group B) were either attached to the skull base or in the middle of the posterior fossa.

Tumour volume was estimated by measuring the maximum diameters based on the three-dimensional measurements in post-contrast CT and MR images; the final volume was calculated by using the formula $V=4/3\pi\ (a\times b\times c)/3$, where a, b, and c represent the three maximum diameters [11]. The extent of peritumoural oedema was determined using MR images and brain CT. The oedemas were identified either as hypodense areas around the tumour in brain CT or as hyperintense signals on T2-weighted MRI. The extent was graded as GR0, GR1, or GR2 [28]. GR0 represents either the absence of oedema or the presence of a small halo around the tumour. GR1 represents an oedema extending variably along the tracts of the white matter but not infringing on the whole hemisphere. GR2 represents holohemispheric or nearly holohemispheric oedema.

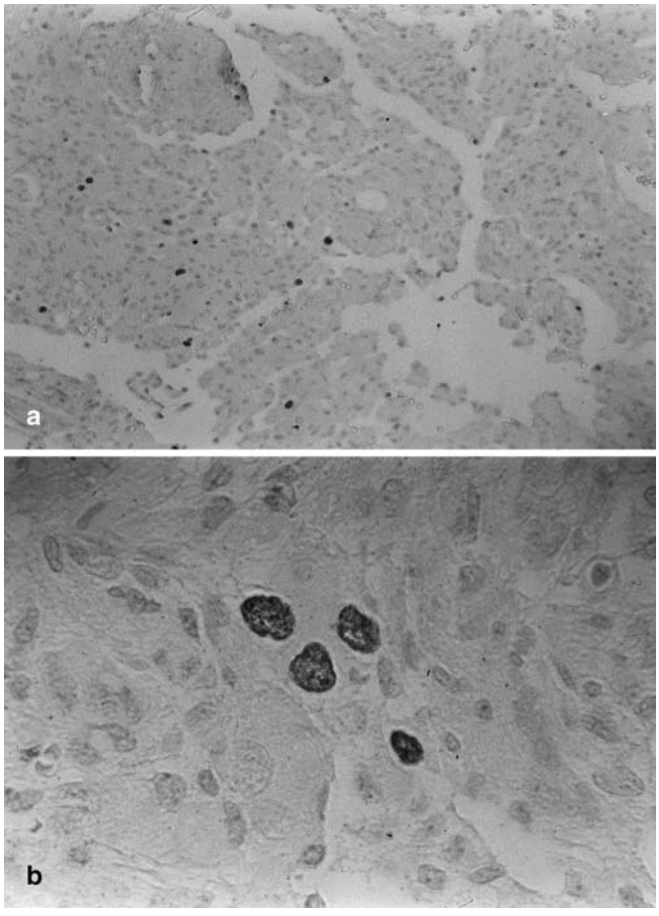

Fig. 1a, b MIB 1 immunostained meningioma cells fixed in formalin and paraffin-embedded. The positive nuclei of proliferating cells strongly show dark brown contrast (DAB as chromogen). LI=5%. **a** Original magnification ×100. **b** Original magnification ×400

Immunohistochemistry

Tissue removed during surgery was set in 4–5 mm, formalin-fixed paraffin sections collected on histological slides coated with 3 aminopropylmethoxysilane. For routine examination, the sections were stained with H&E. Immunohistochemistry was performed on sections next to those used for diagnosis using the instructions in the universal peroxidase kit (Immunotech, Marseilles, France). The primary antibody MIB-1 (Immunotech) was applied to the sections after microwave processing [13]. In short, sections were deparaffinised, treated with 0.03% hydrogen peroxide in methanol, incubated with normal serum, and then incubated with a citrate buffer. Thereafter they were exposed three times to a microwave oven for 15 min each. This was followed by incubation with the primary antibody, the secondary antibody, and the streptavidin-peroxidase complex. The chromogen used was diaminobenzidine (DAB) of aetilcarbazolamine (AEC) (Fig. 1). The sections were then counterstained with haematoxylin. For each series of reactions, a negative control was performed omitting MIB-1.

Cells were considered positive when either the entire nucleus or a portion of it was found to be positive. The numbers of positive and negative tumoural cells were evaluated using high-power magnification (×400) and varied between 1,000 and 1,200 [70]. The

labelling index was calculated using the proportion of positive cells to the total number of cells counted; the results are given as percentages. Care was taken to include areas showing maximal numbers of positive cells.

Statistical method

Clinicoradiological and histopathological statistical data on each patient were obtained and stored in a personal computer to verify the correlations between MIB-1 LI, clinicoradiological and histological characteristics. Statistical analysis took place using chi-squared or Fisher's exact tests and, to check continuous variables, Wilcoxon's rank sum test. Sensibility and specificity were analysed using regression logistic procedures to obtain each positive correlation. *P* was considered significant when less than 0.05. The MIB-1 LI was expressed as the medium LI for each group of variables. The average of all maximum LIs of specific cases was correlated in each variable group ± 1SD. Based on the literature, the authors established a cutoff point at 3%, to which the multivariate analysis was compared [47].

Results

Clinical and histological features

There were 38 females and 17 males from 9 to 82 years of age (mean 49.87±16.80). Concerning the extent of surgical removal, 38 patients were graded I, 15 were graded II, and one each case were graded III and IV. Brain invasion was necessary for the diagnosis of malignant meningiomas. If no cortical tissue was present in the examined sections, the diagnosis of malignant meningioma could not be made. Forty-three benign, 11 atypical, and one malignant tumour were found.

Determination of MIB-1 labelling index

The MIB-1 labelling indices (LIs) ranged from 0.00% to 13.00% (mean 2.51±2.89%).

MIB-1 labelling index and clinical features

No definite relationship was established between the MIB-1 LI and age or sex of patients (*P*=0.8155 and 0.768, respectively, chi-squared test). Neurological symptoms in group 1 (classes I and II asymptomatic patients with minor or slight signs and symptoms) were found in 47 patients, with LI<3% in 32 and LI≥3% in 15. Group 2 (classes III and IV patients with major symptoms and signs) consisted of eight patients, with LI<3% in two patients and LI≤3% in six. Mean LI was 2.07±2.51 in group 1 and 5.06±3.78 in group 2. There was a good statistical correlation between MIB-1 LI and intensity of symptoms (*P*=0.043). The severity of symptoms in well-defined symptomatic patients correlated with high MIB-1 LIs, however asymptomatic patients or those with slight symptoms and signs correlated with low MIB-1 LIs (Fisher's exact test).

There was no significant correlation between MIB-1 LI and removal extension grade according to Simpson (*P*=0.483, Fisher's exact test). In Simpson's grade I, 25 meningiomas showed LI<3% and the remaining 13 showed LI≥3%. In Simpson's grade II, eight meningiomas showed LI<3% and the remaining seven LI>3%. In Simpson grade III, only one case showed LI>3% and in Simpson grade IV, and only one showed LI<3%.

MIB-1 labelling index and radiological features

When analysing the anatomical region, no significant correlation was found between MIB-1 LI and location. Nevertheless, when dividing the anatomical regions into groups of supratentorial compartment with no relation to the skull base (group A) and skull base (group B), a significant correlation was found between MIB-1 LI and these groups (*P*=0.026, Fisher's exact test). Group B had significantly higher mean LIs than group A: 3.85±2.44 vs 2.34±2.92. Group A showed 33 tumours with MIB-1 LI<3% and 16 with MIB-1 LI≥3%, and group B showed one tumour each with MIB-1 LI<3% and MIB-1 LI≥3%.

The authors found no statistical correlation between MIB-1 and tumour volume. Thirty-four meningiomas with a mean volume of 40.31 cm^3 (range 1.23–179.21 cm^3, SD 40.31 cm^3) had LI<3.0%, and 21 meningiomas with a mean volume of 55.91 cm^3 (range 2.42–302.37 cm^3, SD=66.22 cm^3) had LI≥3.0% (*P*=0.4984, Wilcoxon 2 sample test normal approximation).

There was a statistically significant correlation between MIB-1 LI and the extent of peritumoural brain oedema (*P*=0.007, Fisher's exact test). There were 28 cases in the GR0 group, 19 in GR1, and eight in GR2. Tumours in group GR2 oedema had a higher MIB-1 LI than those in group GR1, which in turn had a higher MIB-1 LI than those in group GR0. In GR0, 21 tumours were found with LI<3% and seven with LI≥3%. In GR1, 12 tumours were found with LI<3% while seven had LI≥3%, and in GR2 there were one tumour with LI<3% and seven with LI≥3%. The mean LIs in groups GR0, GR1, and GR2 were 1.49±1.62, 2.78±3.36, and 5.43±3.37, respectively. For each unit of peritumoural oedema classification, the chance of MIB-1 LI being higher than 3% increases to 3.312 times (odds ratio) (Fig. 2).The statistical model using peritumoural oedema has a probability level of 0.240, sensibility of 66.7%, and sensitivity of 61.8%.

MIB-1 labelling index and histological grade

The MIB-1 LIs of atypical/malignant meningiomas were higher than those of benign meningiomas, but this relationship had no statistical significance (*P*=0.177, Fisher's exact test). The mean LI of the atypical and malignant meningiomas was 4.10±3.64, and that of benign meningiomas was 2.07±2.52. The authors did

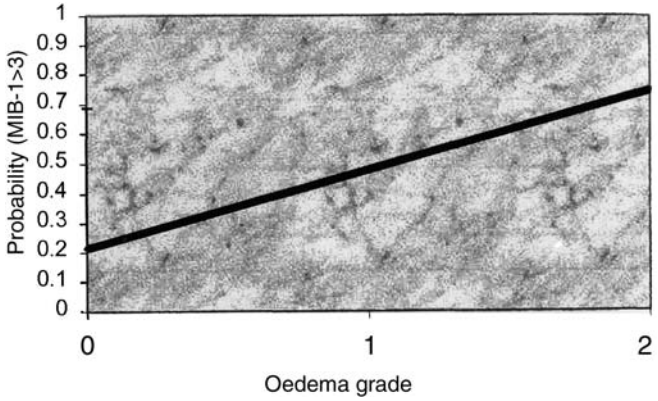

Fig. 2 Linear curve obtained statistically (peritumoural oedema × MIB-1 LI), demonstrating that for each higher peritumoural oedema class, the chance of MIB-1 LI being higher than 3% increases to 3.312 times (odds ratio)

not differentiate between the various types of benign meningiomas. There were 29 benign and five atypical meningiomas with LI<3%, and 14 benign and seven atypical/malignant meningiomas with LI≥3%. Two cases of recurrent meningiomas showed LIs of 2.2% and 3.0%, respectively, and there was no significant correlation between their MIB-1 LIs (P=0.622).

Discussion

Evaluating the proliferative pool in a population of tumour cells is useful when estimating tumour biology and other parameters. In former times, pathologists relied on the mitotic index either as a number of mitotic figures seen in a high-power field or as a relation between the number of mitoses and total number of cells counted. It has been shown that, for some systemic tumours, this index is a reliable criterion for grading malignancy [36]. However, for tumours in the central nervous system, the mitotic index is not sufficient for estimating biological behaviour due to heterogeneity of the cell population and features secondary to technical manipulation which may mask the mitotic figures [40].

Tumours exposed to trytriated thymidine were studied using autoradiographic techniques. Using this method, cells in the S phase incorporate 3 H-thymidine, and the positive nuclei leave Na impressions on the photographic film of the tumour slides [23, 24]. As an alternative, the immunohistochemical localisation of bromodeoxyuridine (BUdR) was used to evaluate the proliferative index. Bromodeoxyuridine is an antagonist of thymidine and therefore is incorporated into the cell at the S phase [60]. These methods presented disadvantages:

1. They had to be infused intravenously prior to surgery
2. Not only the tumour cells but also normal replicating cells were incorporated by the agents
3. The agents occasionally caused myelosuppression [53]

The proliferating nuclear cell antigen (PCNA) was detected in cells in the proliferative pool [27, 34, 50, 63, 75] in tumours, including those taken from the central nervous system. However, many factors seemed to affect PCNA detection when using immunocytochemistry, i.e. dexamethasone therapy used for growth factors [21] and when handling paraffin-embedded tissue.

When determining the nucleolar organiser region, AgNORs were used to access the tumour proliferation index, including meningiomas [39, 40, 63]. Using this method, the associated argyrophilic protein in the nucleolus showed proliferative cellular action. However, this is not directly related to the cell cycle. Argyrophilic nuclear organiser regions are loops of ribosornal DNA in the nucleoli whose proteins are argyrophilic. The difficulty in counting them, considering human variance, proved to be the most important limitation to this method [22, 40, 43].

Flow cytometry was used to estimate ploidy and the cells in the S phase of a given population in the cell cycle, using either unfixed or paraffin-embedded tissue [5, 30]. With this method, the spatial relationships of the cells are lost and cells other than tumoural ones are included [5, 30, 58]. Meningiomas have been studied with flow cytometry [74] and compared with immunocytochemistry and the moAb MIB-1. It was shown that, in recurrent meningiomas, both LI and flow cytometry were higher in recurrent tumours than in nonrecurrent tumours [74].

The moAb Ki-67 was used as a cell proliferation operational marker, due to the initial remarkable paucity of knowledge relating to the protein recognised by this antibody [16, 17], which has been used to estimate the proliferative index in several groups of human tumours, including non-Hodgkin's lymphomas [17], carcinomas [18, 41], melanoma [36], and other large varieties of brain tumours [12, 20, 38, 48, 53, 54, 64, 70, 76]. The percentage of Ki-67-positive cell nuclei was found to reflect the histological grade of malignancy. There is a heterogeneous distribution of Ki-67-labelled cells in meningiomas, which reflects the multicentricity of proliferation [65].

Recently, the antigen Ki-67 was additionally characterised by immunobiochemical and molecular biological procedures. Ki-67 recognises two proteins, 345 kDa and 395 kDa [19, 46], and new moAbs were developed against recombinant parts of the protein, MIB-1, -2, and -3 moAbs [13]. Two of these, MIB-1 and MIB-3, are able to detect the Ki-67 antigen in formalin-fixed and paraffin-embedded sections following microwave oven treatment of the specimens [13]. The interaction between Ki-67 or MIB-1 antibodies and Ki-67 Ag was enhanced in the presence of undergraded DNAs, indicating that DNA modulates the conformation of Ki-67 Ag, and thus the altered conformation of KI-67 Ag is more reactive than the pure protein to both Ki-67 and MIB-1 antibodies [42]. The present authors employed the MIB-1 moAb using immunocytochemistry in this study.

The correlation between LIs obtained by immunocytochemistry with BUdR and MIB-1 was reported as good [56, 74] and supports the use of anti-MIB-1 as an alternative for determining the proliferation index in meningiomas, avoiding the administration of potentially mutagenic and myelosuppressive drugs [56]. In theory, the proliferation indices obtained with Ki-67 and MIB-1 should be equivalent [16], however the two may not be identical because frozen sections are notoriously more difficult to analyse and are usually smaller than paraffin-embedded ones.

It is well known that the tumoural tissue obtained from patients with neurofibromatosis has a higher LI than that found in some non-neurofibromatous tumours taken from the central nervous system [2, 3]. The meningiomas of patients with neurofibromatosis have higher MIB-1 LI than those with sporadic meningiomas [4], as with vestibular schwannomas [2, 3]. Taking this into account, we excluded patients with neurofibromatosis from our study.

The authors could identify no correlation between LI, age, and sex. This has also been demonstrated by other authors [4, 28, 40, 51]. There was no statistically significant difference between LI and isolated symptoms. However, when the patients were divided into asymptomatic and symptomatic classes, a statistically significant difference was found between the two LIs(P=0.0043). Similar results have been described by other authors [4, 51]. It is difficult to analyse the relationship between LI and clinical symptoms in meningiomas, as these are determined by the location of the tumour and associated oedema. It is suggested that rapidly growing tumours are accompanied by varying degrees of oedema, and this may be responsible for the clinical symptoms [31, 32].

No statistical relationship was found between Simpson's grade of resection and MIB-1 LI. Resection depends on microsurgical techniques, surgeon's skills, anatomic location, and the clinical condition of the patient. There is no understandable reason for the proliferation cells to influence the grade of resection and therefore cause difficulty. This fact is confirmed in the literature [14].

The authors found no statistical relationship between LI and different anatomical locations: similar results were observed by Nakasu and coworkers [51]. However, after subdividing the tumours into two groups, those not located in and those attached to the skull base, we found a higher MIB-1 LI in the latter. The invasion in skull base tumours is known and accepted to have a worse prognosis for total removal. However, this particular point remains controversial [8].

Tumour volume showed no correlation with the MIB-1 in our study, and similar results were reported by others [4, 27]. However, Nakasu and coworkers demonstrated a weak but significant correlation [51]. Large tumours may show low MIB-1 LI, probably due to other factors such as nutrition of the cells in the centre of the tumour and limited area of growth. In cerebellopontine angle (CPA) schwannomas and benign extra-axial tumours, Aguiar and coworkers [3] showed an inverse correlation between size and MIB-1 LI in 1995, probably due to restriction of growth depending on cellular contact inhibition and trophic nutritional factors.

In our series, there was a strong relationship between peritumoural brain oedema and increased LI (P=0.007). Eighty-seven per cent of the tumours removed from patients in group GR2 (n=8) had LIs higher than 3%, compared to 37% of tumours in GR1 and 33% in group GR0. Similar results were described in other series [28, 51]. It has been suggested that peritumoural oedema is one of the criteria for aggressive meningiomas [26, 67, 71].

Peritumoural oedema is a specific problem causing difficulty in surgical management of the meningiomas. Extensive brain oedema may cause severe neurological deficits, which limits the surgical field during the approach. However, we have seldom observed the presence of arachnoid in the tumour bed or in areas of softened and oedematous brain tissue, despite careful removal of the tumours. The incidence of peritumoural brain oedema in meningiomas varies between 40% and 78% [26, 67, 68]. Factors that may influence the aetiology of peritumoural oedema include tumour size, histological subtypes, vascularity, venous stasis, type of arterial supply, sex hormone receptors, secretory activity, inflammation (lymphocytes and macrophage infiltrates), and brain invasion [9, 10, 26, 28, 29, 44, 45, 63, 67, 69, 71].

The degree of peritumoural brain oedema as identified by CT scan was found to correlate with the clinical evolution and size of the tumours, whereas the histological features were less significant [44]. Maiuri and coworkers [44] correlated mitosis and necrosis with brain oedema in 92% of cases, but this level was not reported in other series [9]. When studying peritumoural blood flow in intercranial meningiomas, Tatagiba and coworkers [69] observed that, in individual cases, blood flow in the peritumoural oedematous area was very low. Their findings suggest that the hypodense areas surrounding meningiomas does not solely represent vasogenic oedema but may also represent tumour pressure ischaemia [29, 69].

In the present series, we verified that for each increase in peritumoural oedema grade, the chance of LI being higher than 3% rises to 3.312. However, a high LI alone does not seem to be directly responsible for perifocal oedema, because even slow-growing tumours with low LI may exhibit perifocal oedema [51]. The fact that we have not yet verified a relationship between MIB-1 LI and histological grade may be due to the particular growth pattern of meningiomas, which is dependent not only on their proliferative activity but also on other parameters, including cell cycle duration and cell turnover [35].

Histopathological data alone are not reliable predictors of the behaviour and clinical course of meningiomas [66]. In common with our findings, Assieti and coworkers [5], employing BUdR, demonstrated no difference in LI between benign and anaplastic meningiomas. A possible correlation between Ki-67 or MIB-1 and the recurrence of

meningiomas has been previously investigated [58, 70]. According to Roggendorf and coworkers [58], recurrent meningiomas always demonstrate a higher Ki-67 LI. Shibuya and coworkers [62] described that the mean BUdR LI of recurrent tumours was significantly higher than that of nonrecurrent tumours (3.9±2.6% vs 1.9±1.0%, P=0.005) and demonstrated recurrence rates of 100% for tumours with LI of ≥5%, 55.6% with LI of 3–5%, and 30.6% with LI of 1–3%. This is also controversial, as other authors showed no statistical difference between the LI on recurrence and at initial presentation [14, 43].

When studying 106 meningiomas (49 by means of MIB-1), Miyagami and coworkers [47] recently reported a much higher proliferating cell index in nonrecurrent meningiomas than in recurrent meningiomas (10.6±7.7 vs 1.9±1.5). In most of the recurrent meningiomas, LI was higher at the time of recurrence than at the time of initial surgery. We have observed no recurrence since beginning this study, but the follow-up is still short. Prayson [56] concluded that there was no obvious difference regarding MIB-1 index and patient status in the most recent follow-up.

Knowledge of the correlation between the oedema grade viewed in CT or MRI and the proliferation index is more reliable in predicting the biological behaviour and prognosis of tumours before surgical treatment.

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