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Primary Resected Meningiomas: Relapses and Proliferation Markers

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Abstract. *Relapses of meningiomas are a well known phenomenon during follow-up. The significance of sex, age, surgical treatment and mitotic frequency with regard to relapses are still a matter of debate. Patients and Methods: The study included 125 meningioma patients who underwent surgical intervention between 1986 and 1997. They were in follow-up for 3, 5, 10 and 15 years; they were grouped as "stable" or "relapsing" tumours. The follow-up was based on magnetic resonance image (MRI) and tomodensitometry (TDM). The labelling index for Ki67 and PCNA (proliferation markers) was scored at resection. Risk factors for relapse were reviewed using univariate analysis and Cox hazards model. Results: One hundred and twenty-five patients were under medical control of whom 26 showed a relapse. Among them 25 arose from subtotal resected tumours and 1 was a recurrence. Relapses comprised 16 females and 10 males. Tumour relapses at 3,5,10 and 15 years were, respectively, 8.8%, 13.6%, 17.6% and 20.8%. Proliferation markers, at group level, were statistically significantly different to distinguish stable from relapsing and malignant from benign meningiomas. Factors significantly associated with tumour relapse in univariate analysis were incomplete resection, histopathology and proliferation markers. In multivariate analysis the proliferation markers and incomplete resection were the only significant risk factors ($p<0.05$) for relapse. Conclusion: To avoid relapses of meningiomas, total resection is recommended. The resection type and proliferation markers are predictive factors for tumour relapse. The proliferation markers cannot be applied at the individual level.*

Meningiomas, which are mostly slow growing benign tumours, sometimes remain a source of disappointment for the neurosurgeon. Indeed, since the monograph on meningiomas by Cushing and Eisenhardt (1), more recent publications have not added new information about their natural evolution. Radical surgery is the treatment of choice. Even in this case relapse occurs with a rate varying from 32 to 35% at 15-year survey, while relapse rates rise to 82% in subtotal resection in some studies (2,3).

The clinical factors most commonly retained in predicting evolution are patient's age (less than 40 years), male gender, unfavourable tumour location and incomplete surgical resection (4,5). Histological typing of the tumour, apart from malignant or atypical meningiomas, has shown its limitations concerning evolution (6-9).

Cell-proliferation markers studies raised many hopes, but still give contradictory results (10,11).

In the present study of the postoperative tumour evolution of 125 patients in correlation with the proliferation markers Ki67 as MIB1 and PCNA, clone PC10 is presented. So the goal of this study was to assess the predictive value of the different risk factors and especially the proliferation markers at group and at individual level. These might lead to elements for more rational treatment planning and follow-up.

Patients and Methods

Study description. This was a retrospective study of patients with meningiomas operated on in our Neurosurgical Department by the same surgical team, between 1986 and 1997, with confirmed histopathological diagnosis. The resection was classified following the Simpson's classification (12).

In a second step, the resection types were grouped as "*macroscopic total resection*"(MTR) including Simpson's grade I or II, "*subtotal resection*"(STR) Simpson grade III or IV and grade V. One hundred and thirty-nine patients were initially introduced, but only 125 could be followed in the current study. None of the patients was known to be suffering from neurofibromatosis type 2 (NF2). The follow-up included TDM and/or MRI data.

Histopathology and proliferation markers. The histopathological diagnosis was done by two independent histopathologists. After resection the tumour was formalin-fixed and paraffin-embedded for routine histology, which included H&E stain, Trichrom Masson

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Key Words: Meningiomas, proliferation markers, risk factors, relapse, recurrence, regrowth.

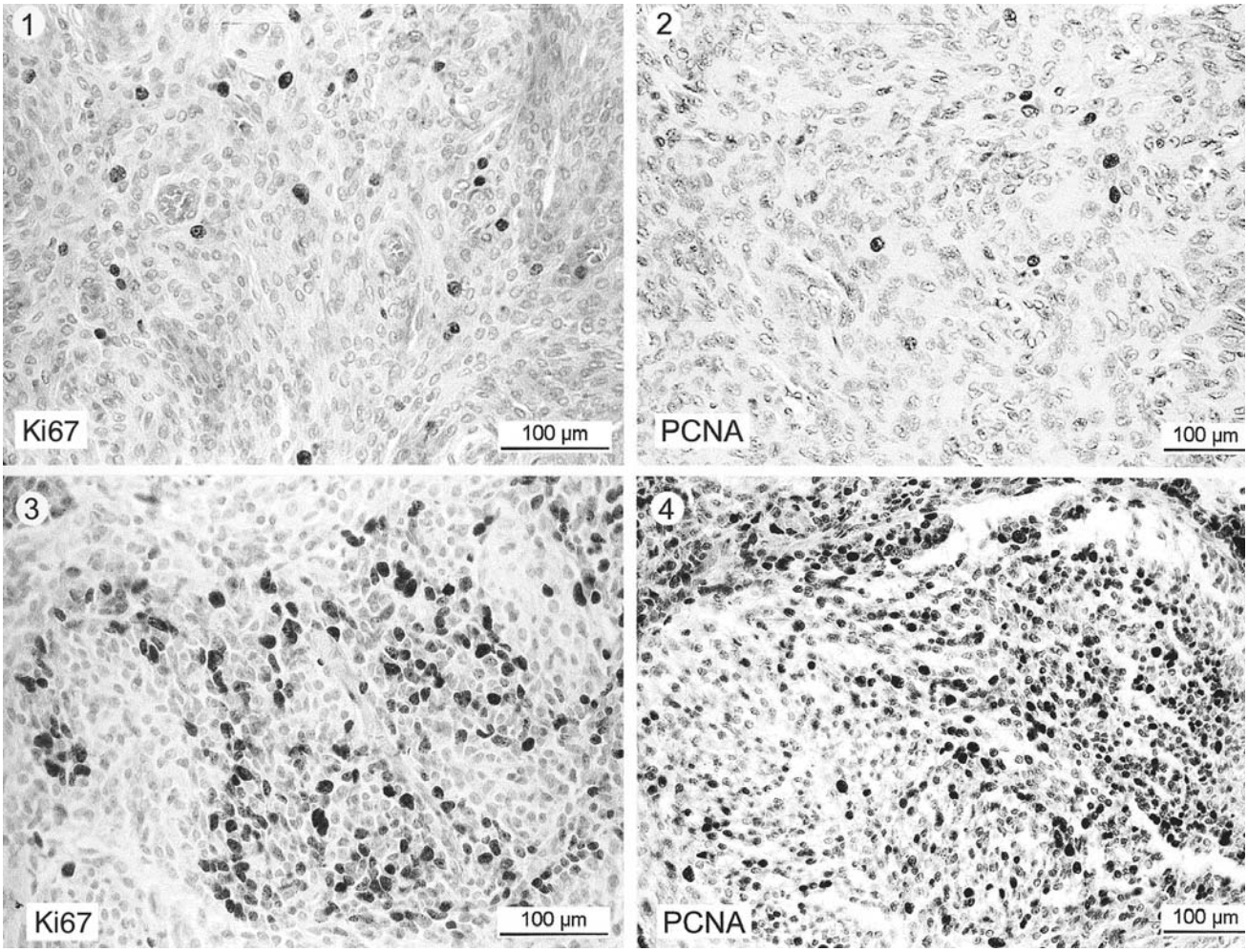

Figure 1. *Immunohistochemical features of benign and malignant meningioma. Peroxidase-antiperoxidase stain for the proliferation markers Ki67 and PCNA. 1 and 2, cells stained by Ki67 and PCNA in a benign tumour. 3 and 4, cells stained by Ki67 and PCNA in a malignant tumour.*

stain for connective tissue, GFAP to exclude glial tumours and vimentin to confirm the mesenchymal origin of the meningiomas.

Special interest was paid to the proliferation markers Ki67 (MIB1) (13) and PCNA of the PC10 clone (14). The staining and counting of the different groups is done after tumour resection and not on archival material. The counting was done at regions of the highest labelling by 3 observers on 200 tumour cells (Figure 1).

The WHO classification (9) gave the following tumour distribution at the beginning of the study: 63 meningothelial, 35 fibrous, 29 transitional, 2 angiomatous (WHO grade I) and 10 malignant and atypical meningiomas (WHO grade II and III).

Follow-up. The evolution (stable or relapse) was evaluated at four consecutive time-intervals, respectively, after 3, 5, 10 and 15 years (or more). The follow-up included essentially magnetic resonance image (MRI) and sometimes tomodensitometry (TDM). The remnants of the original tumour, not changing macroscopically at radiological control or with lack of evidence for tumour relapse, were referred to as *stable*. *Relapse* was defined as both regrowth and recurrence. *Regrowth* was used when remnants of the tumour enlarged. *Recurrence* was when a tumour, having been macroscopically and microscopically totally removed, appeared again.

Statistical analysis. Values were expressed as mean±SD and as percentage when appropriate. The major response variable used in the analyses was tumour relapses within the follow-up period. Both groups were compared by the Mann-Whitney *U*-test for continuous variables and by the Fisher-exact or Chi-square tests for categorical variables. Relapse-free survival probabilities were calculated by the Kaplan-Meier method; comparison between relapse-free survival probabilities of different groups was performed by the log-rank test. The association of these variables with tumour relapse was evaluated with multivariate analysis using Cox proportional-hazards regression models. All variables were put into models: age, gender, tumour location, tumour extension, histopathology, resection, Ki67 and PCNA. Since resection is dependent on the tumour location, we put an interaction term

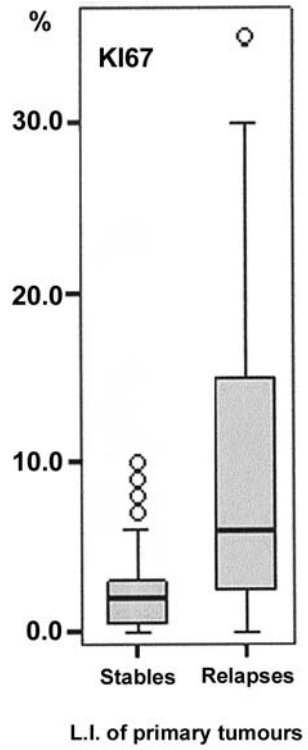

Figure 2. *Ki67 labelling index. Statistical difference (p<0.0001) between stable and relapses.*

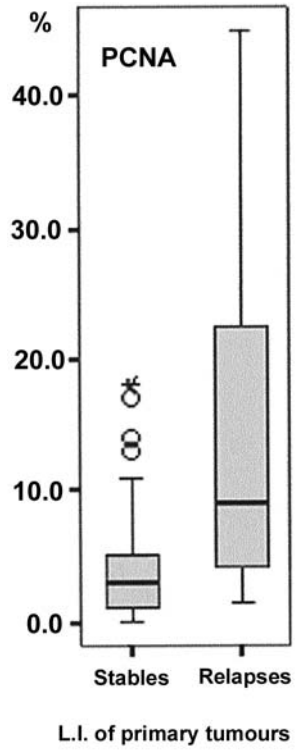

Figure 3. *PCNA labelling index. Statistical difference between stable and relapses (p<0.0001).*

between these two variables in the model, which proved not to be statistically significant ($p=0.380$ for the Ki67 model and $p=0.299$ for the PCNA model). For the variable tumour extension, "no extension" was used as reference. All reported *p*-values are two-tailed. When appropriate, hazards ratio (HR) and 95% confidence intervals (95%CI) are reported. All statistical analyses were carried out with SPSS11 (SPSS Inc, Chicago,IL, USA).

Results

Operative data. A total of 170 operations were performed, including 139 first interventions and 31 reoperations.

First operation: At the first operation, a Simpson grade I resection was done in 32 cases (23%) and grade II in 63 cases (45.4%), meaning 68.4% macroscopic total resection (MTR); grade III in 31 (22.3%), grade IV in 12 (8.6%) and, finally, in only 1 case (0.7%) a grade V resection (biopsy), accounting for 30.9% subtotal resection (STR).

Reinterventions: At reoperation these were, respectively, 13% (grade I), 34.8% (grade II), 17.4% (grade III) and 34.8% (grade IV).

The perioperative death (during operation or within the following 30 days) rate was 2.9% for the first operation and 10% at reoperation. The mean age of perioperative death was 69.6 years at first operation, lowering to 37 years at reoperation. The complication rates (neurological) were 23.7% at the first operation, most of them being transient, and 48.4% at reoperation.

Table I. *Characteristics at the first operation.*

Variables	Stable(n=99)	Relapse (n=26)	*P* value
Gender (male)	24(24.2%)	10(38.4%)	0.214
Age, yr	56(7-84)	51(25-71)	0.109
Tumour location			0.120
Favourable	63(63.6%)	12(46.1%)	
Unfavourable	36(36.3%)	14(53.8%)	
Tumour extension			0.519
None	68(68.6%)	16(61.5%)	
Dura	11(11.1%)	2(7.6%)	
Other tissues	20(20.2%)	8(30.7%)	
Resection			<0.0001
Complete	77(98.7%)	1(1.3%)	
Incomplete	22(46.8%)	25(53.2%)	
Histopathology			0.017
Benign	97(97.9%)	22(84.6%)	
Malign	2(2.2%)	4(15.3%)	
Ki67	2.4(0-10)%	10.4(0-35)%	<0.0001
PCNA	3.8(0-18)%	13.9(1.5-45)%	<0.0001

Table II. *Multivariate analysis (Cox's model). Significant predicting factors for tumour relapse.*

Model A with Ki67. Variable	Hazard ratio	95%CI	*p*-value
Ki67	1.07	1.06-1.14	0.001
Resection	10.1	2.8-35.2	<0.0001
Model B with PCNA. Variable	**Hazard ratio**	**95%CI**	***p*-value**
PCNA	1.06	1.03-1.10	<0.0001
Resection	13.7	3.11-60.8	0.001

Proliferation markers. Ki67 (13), a nuclear antigen of uncertain proliferative function, is detectable in cycling cells from G1- to M-phase and PCNA (14), a member of the cyclin family, is generally activated in G1- and S- phase. Both markers were demonstrated in the freshly dissected tumours at their first resection (Figure 1).

Statistical analysis demonstrated a significant difference in labelling index (L.I.) between the stable tumour group and the relapse group for Ki67 (Figure 2) and for PCNA (Figure 3) (for both $p<0.001$). The difference was also significant between the group of benign meningiomas and the malignant ones. (Ki67 $p=0.009$; PCNA $p=0.001$)

These data provide a clear-cut statistical difference between benign *versus* malignant meningiomas and stable versus relapsing meningiomas at group level. Some caution, however, is necessary concerning the statistics for patients in the malignant group, due to their limited number .

Follow-up and tumour relapse. In the cohort of 139 patients, 4 died during the first operation, 2 were lost at control and 8 died from other reasons before the first evaluation at 3 years. Among the remaining 125, 26 patients (20.8%) had a tumour relapse, with 25 being a regrowth and 1 recurrence. Among the regrowths, 3 were multicentric at the time of first operation. Ninety-nine patients (79.2%) remained unchanged or stable. Regrowths were seen in 53.2% of the subtotal resection patients.

The mean age of the patients without tumour evolution was 56.3 years (range 7 to 84 years). For the relapses it reduced to 51.3 years mean (range 25 to 71years). The sex ratio for those relapsing was 10 males (38.5%) to 16 females (61.5%). In the "stable" category were 75 females (75.8%) and 24 males (24.2%). Following the WHO (9) classification, 26 of the relapsing tumours included 8 malignant. The number of tumour relapses ranged from 1 to 4 (mean of 1.3). The relapse interval ranged from 5 to 208 months (mean 69.0 months). The relapse rates of the 125 patients in follow-up estimated at 3, 5, 10 and 15 years were successively 8.8%, 13.6%, 17.6% and 20.8%.

Tumour relapse risk factors. For univariate analysis, see Table I.

As regards *multivariate analysis* among different variables, only proliferation markers (Ki67, PCNA) and resection were identified as significant ($p<0.05$), predicting risk factors for relapse in different models. Yet as both Ki67 and PCNA were introduced in the model, no risk factors were significant (Table II).

Discussion

Meningiomas are estimated to represent 13% to 26% of primary intracranial tumours (15). There is a clear sex predomination for the female gender with a sex ratio of 3:2, or even as much as 2:1 in other series. Our results confirmed the 2:1 ratio: 96 (69%) females to 43 (31%) males. Male sex has been associated by some authors with a higher recurrence potential (16) or with malignant form (15,17). This was not confirmed here on univariate analysis ($p=0.214$).

In our series, of the 26 relapses 16 (61.5%) were females and 10 males (38.5%), while concerning the regrowth of 25, we had 15 females and 10 males. The only recurrence was a female with probable radio-induced meningioma.

This predominance of females might point to the effect of oestrogen and progesterone on the proliferative capacity of meningial cells. *In vitro* experiments (18) support the idea that meningioma-derived cells have a higher proliferation rate in the presence of female hormones than without.

Meningiomas are encountered mostly in the sixth decade, as in our series. The mean age was 56 years for all the series but fell to 51.3 years for the relapse group. Though age was not significant ($p=0.109$) in this review, different series have shown young age as being a factor for a reduced progression-free survival (16,19,20). This might be linked to the limited number of patients in the series. The ideal treatment of intracranial meningioma still remains today, as stated by Simpson (12) more than forty years ago, to be a complete surgical tumour removal with the involved dura and all other invaded tissues. More specifically for the dura mater, a generous margin whenever possible should be taken (21,22). This principle, however, is in practice dependent on the characteristics of the tumour, among which principally are tumour location, consistency, size and eventual encasement of neural or vascular structures. The age and the medical condition of the patient are other important factors (23).

Multivariate analysis of our series identified only resection type and proliferation markers as significant risk factors for relapse

Resection. The aim of meningial surgery should be a total or a radical tumour resection. The surgeon has to be meticulous but, when facing a malignant lesion, he should

be more aggressive, prompting some authors to recommend a more radical operation (17).

Concerning complementary therapy, in our series benign lesions were not treated with ionising irradiation, with the exception of relapses to avoid radio-induced damage (24), as seen in the only recurrence case in our series. In cases of malignancy, irradiation was given to a total dose of 5400-6000cGy in a 6-week period by classical external therapy or by single dose with radiosurgery. Chemotherapy is not included in the standard treatment.

So the complete surgical removal of the tumour is the important step to overcome relapses during follow-up, as clearly shown in our series (Figure 4).

Multicentricity in 3 cases of our group has to be considered as being part of partial resection. In those cases the proliferative capacity of the different tumour masses was the basis of the relapse. Multicentricity can also be observed at the first resection or may arise after initial resection. This argues against dissemination by surgical intervention as proposed by some authors (25).

In comparison to the data from literature, the low number of recurrences in our series might have depended on the definition of recurrence. In our series the surgical total removal was completed by a histological evaluation of the tumour in which the border zone of the normal tissue was tumour cell free, so the theoretical possibility of regrowth of some cells left over at the first operation was minimal. Macroscopic complete removal might be misinterpreted by the surgeon as he can overlook distant foci of meningioma cells and infiltrated cells in bone and the sinus wall. Also the proliferation rate of meningioma cells is a fundamental factor, even with one cell left in place (8) after intervention. As suggested by Jaäskelainen *et al.*, if a meningioma doubles in 1000 days, it would take 11 years to be visible on a CT scan when you have 1 dividing cell left at intervention.

Proliferation markers. Our results demonstrate a clear-cut difference in proliferative activity between benign and malignant meningiomas as well as between stable and relapsing for PCNA and for Ki67.This relation was shown 15 years ago by Roggendorff (26) and others (27) for Ki67 and for PCNA by Cerda *et al.* (28) separately.

The data on regrowth, which means relapse after partial resection, are as follows: of 47 tumours with only a partial resection as treatment, 25 (53.6%) demonstrated a regrowth after 15 years, which suggests that probably the proliferation rate of the tumour or its remnant is the driving force of the regrowth. From this point of view it might be deduced that proliferation markers can help the surgeon to estimate the evolution of a tumour. The higher the proliferation rate in the resected tumour tissue, the higher the probability of relapse. Yet proliferation counting as such has some inherent factors of bias *e.g.* sampling or heterogeneity of the tumour, used observation method and examiner competence (4).

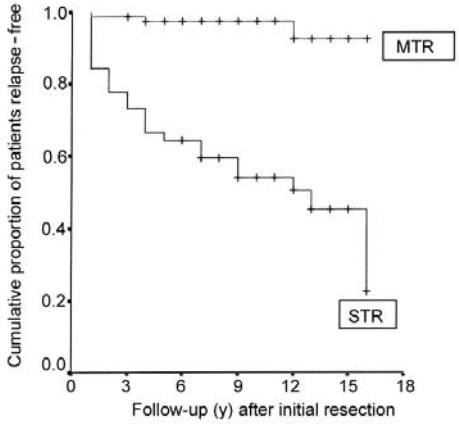

Figure 4. *Curves illustrating the cumulative proportions of patients free of tumour relapses following the resection type (MTR: Simpson I and II; STR: Simpson III, IV and V). Difference between the two groups is statistically significant (log-rank test, $p<0.0001$).*

The proliferating rate in the recurrent case seems to be similar to that of the initial lesion as being up to now growing slowly. A regular follow-up is used in this case. In the literature the discussion on the labelling indices for relapses is still a matter of debate (29,4). On the other hand the definition of relapse, recurrence and regrowth are not standardized in the literature (30,31).

Conclusion

In the presented series of 139 meningiomas, the sex ratio was 43/96, male/female ratio. The series included 70% (n=98) macroscopic total resections and 30% (n=41) subtotal resections.

Relapses were seen in 26 patients of the 125 in follow-up after 15 years. All relapses were regrowths after subtotal resection, with the exception of one recurrence probably induced by irradiation.

So the treatment of choice to avoid relapse still remains total resection, as multivariate analysis identified only resection type and proliferation markers as risk factors for relapse.

The proliferation indices measured by Ki67 and PCNA clearly demonstrate a statistically significant different mean L.I. between relapses and non-relapses and between the malignant and the benign meningiomas.

At an individual level, however, no clear-cut borders in L.I. were observed. So the neurosurgeon cannot rely only upon the individual proliferation data for each patient. Since the mean for the L.I. in putative relapses is higher than in non-relapses, the L.I. can be an important indicator

from the first resection for the neurosurgeon. A strict follow-up after resection in those cases is recommended, particularly in cases of subtotal resection.

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