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Can Recurrence of Meningiomas be Predicted?

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Abstract. *After resection of meningiomas the clinical evolution remains problematic, as no clear-cut predictive criteria are available. In vitro evaluation of meningiomas might help to predict their evolution in vivo after resection. For this goal a confrontation model was tested. A group of 105 patients operated for meningiomas between 1986 and 1997 were reviewed at 3,5,10 and 15 years for tumour evolution by tomodensitometry or magnetic resonance. At operation a fragment of these resected tumours was explanted for cell culture and was confronted with embryonic chick heart as a host tissue. The confrontation between tumour- derived cells and host tissue resulted in three different patterns: respectively a regressive, a non-invasive and an invasive pattern. Resection type, proliferation markers (Ki67 and PCNA) and in vitro confrontation patterns were significant ($p<0.05$) factors in predicting the postsurgical evolution of meningiomas. No correlation was found between proliferation markers and the behaviour in vitro, but invasion in vitro was strictly correlated with recurrence and malignancy of meningiomas.*

Meningiomas can be considered as benign tumours since their clinical outcome after total resection normally leads to complete healing. However, some tumours cannot be completely resected and a number of these partially resected meningiomas might show relapses and become even clinically malignant. This clinical malignancy is, in a number of cases, not correlated strictly with the histopathological criteria of malignancy (1).

The biological behaviour of meningiomas after resection is a clinical problem for which no clearcut predictive criteria are available at the moment. The histopathological analysis on fixed material can be completed by *in vitro* methods that might give information on the viability, proliferation and motility of meningioma cells and, as such, on their clinical evolution.

The *in vitro* behaviour of 117 meningiomas derived from 105 patients, who were operated on between 1986-1997, was evaluated. Primary cell cultures from freshly dissected meningiomas were prepared and, at confluency, these viable cells were allowed to form three-dimensional spheroids. The spheroids of tumour-derived cells were confronted with spheroids of normal tissue.

The objectives of this study were two-fold: first, to study the interaction between meningioma-derived cells and normal tissue *in vitro*; second, to compare the *in vitro* behaviour with the postsurgical evolution *in vivo* with special attention to relapses. The post surgery period covers fifteen years.

Patients and Methods

Patients. One hundred and thirty-five patients with a meningioma (94 females and 41 males) underwent surgical intervention by the same team. One hundred and seventeen of these resected meningiomas were successfully cultured *in vitro*. The Ethics Committee of the University Hospital, Ghent, Belgium, approved the protocol (project 2001/58) for the *in vitro* experiment.

One hundred and five (77 females and 28 males) patients, being operated on for the first time between 1986 and 1997, are in follow-up.

Histopathology and proliferation markers. The histopathological evaluation included a routine stain with hemalum-eosine and a Trichrome Masson for connective tissue. GFAP was applied to exclude glial tumours and vimentin to confirm the mesenchymal origin of the meningiomas. As proliferation markers, the Ki67 and PCNA labelling index (LI) were scored. Ki67 (2) is a nuclear antigen of uncertain proliferative function detectable in cycling cells from G1- to M-phase. PCNA (3), a member of the cyclin family, is activated in G1- and S- phase. Counting was done at the region of the highest labelling by three observers on 500 tumour cells.

According to the WHO classification (1) for meningiomas, the following subclasses were found at first operation: 55 meningothelial, 6 fibrous, 18 psammomatous, 20 transitional, 3 angiomatous (WHO grade I) and 3 malignant /atypical meningiomas (WHO grade II and grade III).

Primary cells cultures. After resection the tumour was divided into two parts, one for histopathological diagnosis and the other for cell culture. The latter was chopped in 1x1x1 mm fragments and

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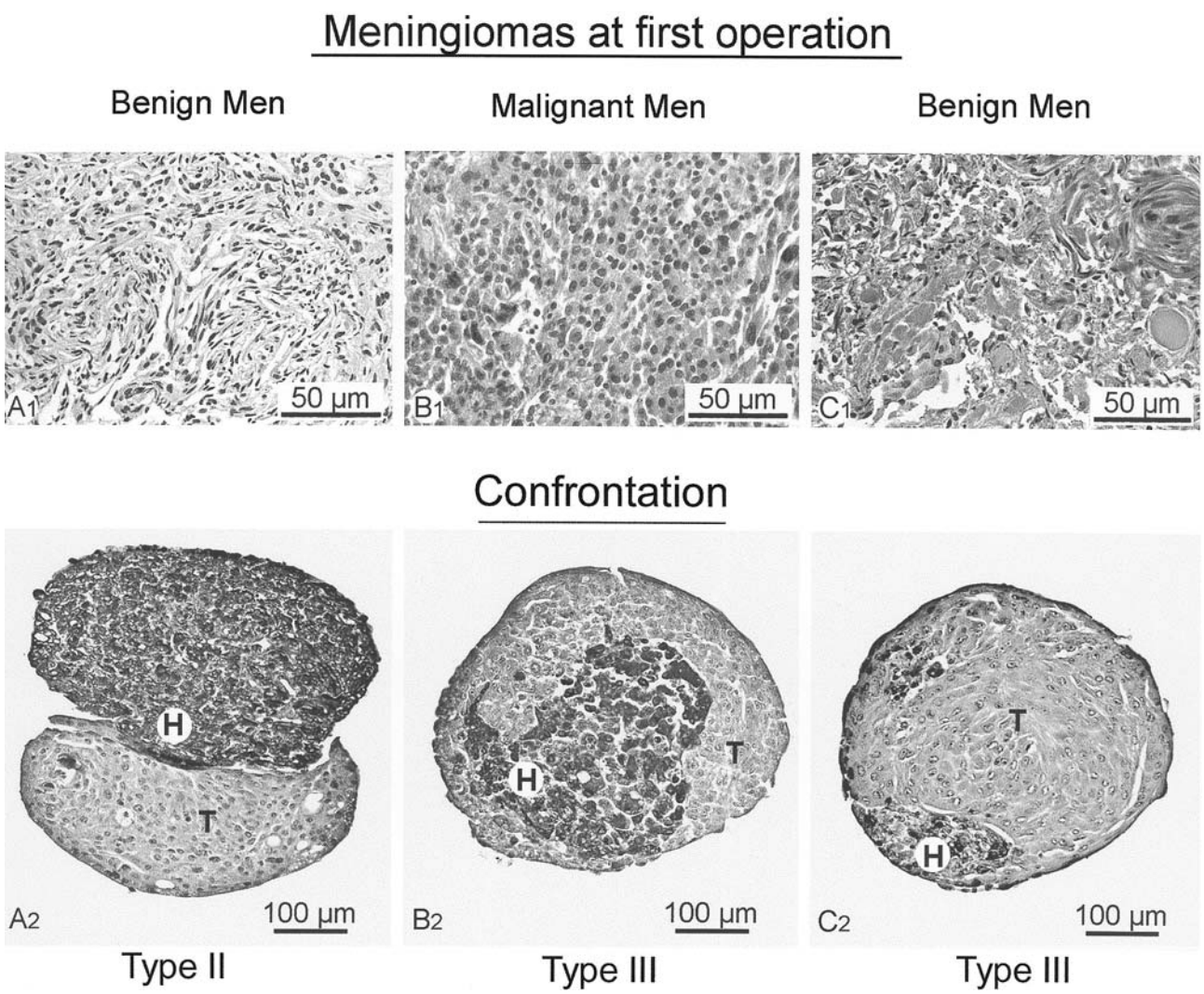

Figure 1. *Histology versus confrontation type.*
A1. Histology of benign meningioma.
A2. Confrontation of meningioma-derived cells (T) with host tissue (H) after 4-day incubation. No invasion of tumour derived into the host tissue. Type II confrontation.
B1. Histology of malignant meningioma.
B2. Confrontation of tumour cells derived from a malignant meningioma (T) with host tissue (H) after 4-day incubation. Invasive pattern of tumour-derived cells into the host tissue.
Type III confrontation.
C1. Histology of a benign meningioma.
C2. Confrontation of tumour cells derived from a histopathological benign tumour (T) invading and replacing the host tissue (H). The tumour cells derived from a histopathological benign tumour behave like invasive cells, malignant cells, in vitro.
Type III confrontation.
A1-B1-C1 stained by H&E; A2-B2-C2 immune stain by chick antibody.

transferred into a 25cm^3 Falcon plastic vessel filled with growth medium and incubated at 37˚C to form a primary monolayer culture. The culture medium was composed of Eagle's medium (Minimal Essential Medium supplemented with Earle's salts) and 10% foetal calf serum. One vol% of a 400mM L-glutamine solution was added. Near confluency cell clusters were scraped off with a rubber policeman and transferred to a non-adhesive agar substrate.

Spheroids and confrontations in vitro. As previously described by Mareel *et al.* (4), precultured heart fragments and tumour-derived cell aggregates were placed in close contact with each other on the surface of semi-solid agar medium (0.4%) in Tyrode. After adhesion of the tumour-derived spheroids to the heart fragments, the spheroid confrontation cultures were transferred into 5ml Erlenmeyer flasks filled with 2ml MEM supplemented with 10% fetal calf serum. The

Table I. *Patients and tumour characteristics at first operation.*

Variables	Stable (n=82)	Relapse (n=23)	*P*-value
Gender (male)	19(23.1%)	9(39.1%)	0.18
Age, yr	56(14-84)	50(25-71)	0.07
Tumour location			0.05
Favourable	54(65.8%)	10(43.4%)	
Unfavourable	28(34.1%)	13(56.5%)	
Tumour extension			0.64
None	54(65.8%)	15(65.2%)	
Dura	19(23.1%)	4(17.3%)	
Other tissues	9(10.9%)	4(17.3%)	
Resection			<0.001
Complete	63(76.8%)	6(26.1%)	
Incomplete	19(23.1%)	17(73.9%)	
Histopathology			0.12
Benign	81(98.7%)	21(91.3%)	
Malign	1(1.2%)	2(8.6%)	
Confrontation			0.02
Regressive	21(25.6%)	1(4.3%)	
Non-invasive	48(58.5%)	14(60.8%)	
Invasive	13(15.8%)	8(34.7%)	
Ki67	2.3(0-10)%	8.7(0-35)%	0.001
PCNA	3.8(0-17)%	12.8(1.5-45)%	<0.001

flasks were shaken at 110rpm under a steady gas flow of 95% air and 5% CO_2 at 37˚C (5).

After 1,2,4 and 7 days of incubation, the cultures were fixed and stained with H&E and chick antibody. After sectioning, the results were classified into three patterns (6); respectively, the regressive pattern (type I), non-invasive pattern (type II) and invasive pattern (type III) (Figure 1). Regressive means the tumour-derived cells do not survive in confrontation. Non-invasive means the tumour-derived cells survive in confrontation but do not infiltrate into the heart fragment and are separated from the heart tissue by a clearcut border. Invasive type means the tumour-derived cells infiltrate into and progressively replace the heart tissue.

Clinical evolution of the patients. The follow-up was based essentially on a magnetic resonance image (MRI) and in some instances on a tomodensitometry (TDM) respectively at 3, 5, 10 and 15 years after surgery. The patients were grouped into two subgroups. The "stable group" comprised patients after total resection and patients with tumour rests not macroscopically changing at radiological control. The second group included the "relapses", which means patients with a regrowth (*i.e.* tumour rest enlargement) or a recurrence (*i.e.* tumour having been totally resected appearing again at the same or at another place). The resection type was classified using a modified Simpson's classification (7). The Simpson class I and II was quoted as "macroscopic total resection" (MTR); a Simpson class III, IV and V was notated as "subtotal resection" (STR).

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. Logistic regression analysis was used to assess the multivariate relationship between multiple patients, and between tumour characteristics and the confrontation test to assess the probability of tumour relapse. For this analysis the regressive pattern was used as reference. To assess the relationship between a continuous variable and the outcome and subsequently to analyze whether a continuous variable needed to be transformed or categorized, we used a smoothing scatterplot (LOWESS) and the method of quartiles for each model. Stepwise forward and backward elimination were used. All covariables which were statistically significant at $p=0.25$ level in the univariate analysis or that were clinically relevant according to previous reports were included in the logistic regression model. We further analysed the correlation between our test and proliferative markers (Ki67, PCNA). All reported *p*-values are two-tailed. When appropriate, odds ratio (OR) and 95% confidence intervals (95%CI) are reported. All statistical analysis were carried out with SPSS11 (SPSS Inc, Chicago,IL, USA).

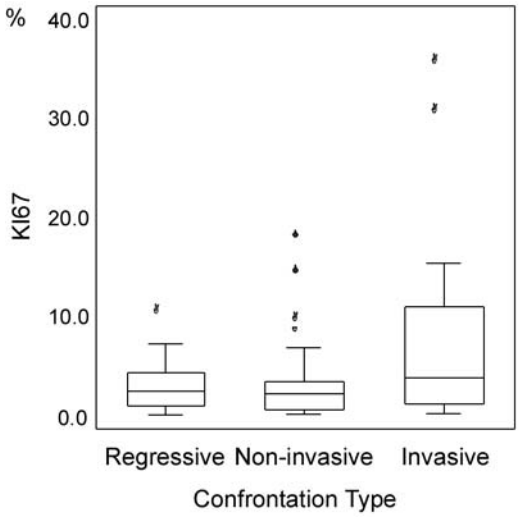

Figure 2. *Ki67 labelling following the three confrontation patterns.*

Results

The results included univariate and multivariate analysis, the correlation between the labelling index for PCNA and Ki67 in the resected tumour mass at the first intervention and the confrontation test. The primary cell cultures, the confrontation patterns and the clinical follow-up, focused on relapses are reviewed too.

I. Univariate analysis. Table I summarizes the results for patients and tumour characteristics at first operation.

II. Multivariate analysis. Besides the regressive, non-invasive and invasive categories of our confrontation, all variables with a *p*-value of < 0.25 according to the univariate analysis, as well as a variable previously reported as relevant, were included. These variables were gender, age, resection, histopathology, Ki67, PCNA, tumour extension and tumour location.

Table II. *Multivariate analysis: impact in predicting meningioma relapse using confrontation test.*

Model A: Without Ki67 and PCNA

Variable	Odds Ratio	95%C	*P*-value
Resection (STR)	10.88	3.51-33.66	<0.001
Confr. Non-invasive Type	7.58	0.85-67.17	0.069
Invasive Type Confr.	18.12	1.47-188.2	0.015

Model B: Including Ki67 and PCNA

Variable	Odds Ratio	95%C	*P*-value
Resection (STR)	6.03	1.29-28.17	0.022
Ki67	1.24	1.02-1.51	0.025
PCNA	1.18	1.01-1.38	0.032

Table III. *Confrontations vs clinical evolution.*

Confrontation	Stable	Regrowth	Recurrence	Total
Regressive	21	0	1	22
Non-invasive	48	12	2	62
Invasive	13 *	8	0	21
Total	82	20	3	105

*MTR (Simpson I&II)

Logistic regression analysis, considering relapse as dependent, was conducted. A first model was built without the proliferation markers. In this case the resection type and confrontation test are significant.

When considering all the variables, only the resection type and proliferations markers were significant in predicting relapses (Table II).

Model B predicts 97% of the stable tumours correctly, while this falls to 53.3 % for the relapse group. At the same time Model A, if less predictive for the stable group with a correct prediction in 85.4%, reaches a predictive capacity of 73.9% in the relapse group.

III. Correlation between proliferative markers and the confrontation test. The Ki67 and PCNA L.I. (labelling index) were studied for the three confrontation groups. Although the mean for both Ki67 and PCNA was higher in the invasive group, no significant statistical difference was achieved (Ki67 $p=0.07$ and PCNA $p=0.45$). The regressive pattern group (n=22) demonstrated a mean Ki67 L.I. of 2.9 and PCNA L.I. of 5.1. The non-invasive pattern group (n=62) had a mean Ki67 L.I. of 2.7 and PCNA L.I. of 4.7. In the invasive pattern group (n=21) the mean Ki67 L.I. was 8.0 and PCNA mean was 8.6. No correlation was found between the proliferation markers and the confrontation type (Figure 2). The correlation coefficient according to the Spearman's test was 0.073 ($p=0.488$) between Ki67 and the confrontation test. Between PCNA and confrontation this correlation coefficient was 0.049 ($p=0.648$), while it was 0.518 ($p<0.001$) between Ki67 and PCNA.

Table IV. *Meningiomas evolution.*

	Benign	Malignant	Total
Regrowth	15	5	20
Recurrence	3	0	3
Relapses	18	5	23

Primary cell culture. Of the 137 tumour fragments collected, 117 (85.4%) were cultured as primary cultures. At confluency, from the 117 primary cultures monolayer flaps were transferred into a shaker and all formed spheroids.

Confrontation results and clinical evolution. As mentioned above, 117 confronting cultures were available. However, at the first clinical and radiological evaluation moment after 3 years, 9 patients could not be classified (stable or relapse). Two were lost at control and seven had died from other causes than their meningial tumour. Three more were excluded due to uncertain diagnosis. Therefore the following data concern a series of confrontations (Table III) concerning 105 patients, comprising 77 females (73%) and 28 males (27%).

In 22 cases, the confrontations were classified as expressing a "regressive pattern" (Type I). They included only benign meningiomas (WHO grade I). The average follow-up period was 11.4 years. The clinical evolution never showed malignant transformation of these tumours and only one showed a recurrence. After verification this appeared to be a multifocal radio-induced meningioma (RIM). Apart from the RIM recurrence case all others tumours, even after STR, showed no relapse.

In 62 cases the confrontations were classified as expressing a "non-invasive pattern" (Type II). The average follow-up period was 10.1 years. Here 48 tumours remained stable and 14 relapsed with 12 regrowths and 2 recurrences (multifocal). As in the first group (type I), no malignant tumours were found here and even no clinical malignant evolution was noted during the cohort observation.

In 21 cases the confrontations were classified as expressing an "invasive pattern" (Type III). Between them 13 were in the "stable" category and 8 relapsed. All of them were regrowths and no recurrences were retrieved. The average monitoring period was 8.8 years . All 13 stable tumours underwent a total removal, a MTR (Simpson I

Table V. *Histopathology and clinical evolution of confrontations expressing an invasive pattern in confrontation at first operation.*

Histopath. at 1st op.	Histopath. at 2nd op.	Clinical evolution
3 benign	3 malign	3 Deceased
2 malignant	2 malignant	2 Deceased
3 benign	3 benign	2 Deceased/ 1 Alive

&II). Analysis of the 8 regrowths revealed that 3 histological benign and 2 malignant tumours remained unchanged, while 3 initial benign tumours progressed towards malignancy.

In summary, for all the series the 23 relapses included 20 regrowths (15 benign tumours and 5 malignant) and 3 recurrences (benign), all of these being multifocal (Table IV).

Discussion

Meningiomas are mostly slow growing tumours arising from the arachnoid cap cells and represent from 13% to 26% (1) of primary intracranial tumours. Females are more commonly affected (female: male sex ratio 3:2 or even 2:1). These tumours are most common in middle-aged and elderly patients with a peak in the sixth decade of life. Neurofibromatosis 2 (NF2) or induced cytogenetic alterations, by radiotherapy, may lead to multifocal localisations of the meningioma. Their monoclonal origin is still a matter of debate. The clinical factors associated with tumour relapse are incomplete surgical resection, linked to unfavourable location or attachment to intracranial structures (8, 9).

As stated by Simpson, total radical resection remains the treatment of choice to prevent relapse and this is confirmed by our data. Supplementary therapy is not standardized. For instance, in which circumstances should a benign meningioma be treated by radiotherapy and to which patients should chemotherapy be administered? The answer is not always obvious because the evolution is sometimes unpredictable, requiring other elements for planning further therapy. In this view the confrontation technique constitutes an interesting tool that might help to predict the *in vivo* evolution.

Primary cell culture-spheroids. This primary culture allows the selection of the more motile, viable and proliferative cells from the tumour sample (10) while necrotic material is eliminated. Putative malignant cells are selected by the *in vitro* culture as they are the most proliferative. The 85.4% primary cell culture success rate may reflect the relative reproducibility of this method for clinical application. The 14.6% failure to grow primary cultures is correlated with different factors such as the presence of dying or necrotic cells in the explanted fragments, the sampling and the medium conditions (10).

Confrontation versus clinical follow-up. The need for accurate prediction of the biological behaviour of meningiomas remains problematic. Histopathology alone proved insufficient (11-13) with the disadvantage of being a "static" examination. The assessment of proliferative capacity by immunohistochemical indicators such as Ki67 or PCNA, though helpful at group level, are not strictly applicable at individual level. However, a high L.I. is *per se* worrisome, particularly in case of incomplete tumour resection. To get a better insight into the biological behaviour of the tumour, the confrontation test seems to be useful and complementary to L.I. and histopathology. The clinical evolution of patients for whom the confrontation expressed a regressive pattern (Type I) did not show a relapse *in vivo* and, as a group, they had a low L.I. (2.9%±2.4) at histopathological analysis. The only exception to this observation was a multifocal radiation-induced meningioma.

The clinical evolution of patients, for whom the confrontation demonstrated a non-invasive pattern (type II) was different from type I. In the type II confrontation (non-invasive pattern) the explanted tumour fragments survived and proliferated without any invasion signs *in vitro*. The tumour-derived cell aggregates developed at one pole of the chicken embryo heart fragment without intrusion at the interface and encircled it progressively without intermingling. The Ki67 L.I. was slightly lower than in the regressive confrontation (2.7%±3.9). The clinical evolution with 13 regrowths confirms the higher proliferation capacity in this group though there is no statistical difference in L.I. between the regressive pattern and non-invasive pattern groups. This suggests the capacity of the confrontation technique to distinguish the tumours by their different biological behaviour *in vitro*. Here too, the lack of invasiveness *in vitro* was confirmed by the absence of clinical aggressiveness during the follow-up. Two multifocal cases were classified as recurrences.

In the invasive pattern (type III confrontation), the tumour-derived cell aggregates proliferated and clearly invaded the host tissue replacing it progressively. The high proliferative capacity is corroborated by the mean L.I. Ki67 (8.0%±10.5) and PCNA (8.6%±12.1), and was higher than in type I and II confrontation. Among the 21 patients in the clinical survey, 13 remained unchanged ("stable") while 8 showed regrowth. All the 13 "stable" tumours were resected completely (Simpson I &II). No relapse occurred. In the 8 regrowths (Table V), after subtotal resection, 3 histologically benign tumours at first

operation progressed towards histological malignancy at regrowth. Three other benign tumours, at first operation, did not change histologically at regrowth, but showed a clinically bad follow-up and 2 of them died. Of the 8 patients with a regrowth only one is still alive. As in confrontation cultures the tumour-derived cells of these 8 patients expressed an invasive pattern, this confrontation model has a predictive value for regrowth. *In vitro,* during primary cell culture, viable and motile cells are selected.

From literature and from our data, there is a distinction between proliferation and invasion as two separate processes (14). If a precise definition of "malignancy" is still debatable (15), what matters for the clinician is to forestall the patient's deterioration and to opt for the best therapeutic choices in his armamentarium. A tumour of aggressive biological behaviour from the clinical point of view is as worrisome as a tumour that is traditionally considered as "malignant" by the histopathologist. Both these aspects are dealt with in this three-dimensional confrontation test.

In summary, the confrontation technique showed here, in the clinical context, its reliability in predicting the biological behaviour of meningiomas. This test might be used to detect patients with high relapse risks, *i.e.* with an invasive confrontation pattern. These could be other than those already identified by histopathology and by proliferation markers as shown by the absence of correlation between these parameters. Finally, all the patients combining STR and an invasive pattern showed a clinical malignant development even when the lesion was classified as benign by the histopathologist. The other patients with a confrontation expresson type III and having had a total resection remained "stable" without recurrence, emphasizing once again the value of Simpson' s work.

Macroscopic total resection is not a guarantee that all meningioma cells are taken away, a minimal infiltration might be overlooked (13) and regrowth might start from these looked-over tumour cells (16).

In conclusion, the certain prediction of the development of a meningioma remains obscure. Combining histopathology and grading, proliferation markers and confrontation will help to solve this problem. By selecting and amplifying *in vitro* tumour-derived cells, confrontation appears to be a valuable tool that deserves to hold a place in clinical practice.

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