Histol Histopath (1993) 8: 1-8

Histology and Histopathology

Clinico-pathological correlations in meningiomas: a DNA and immunohistochemical study

F.F. Cruz-Sánchez[1,2], R. Miquel[1], M.L. Rossi[3], J. Figols[4], A. Palacín[1] and A. Cardesa[1]

[1]Department of Pathology, and [2]Neurological Tissue Bank, Service of Neurology, Hospital Clínico y Provincial, University of Barcelona, Spain, [3]Department of Neuropathology, Midland Centre of Neurosurgery and Neurology, Birmingham, United Kingdom, and [4]Department of Pathology, Hospital Universitario «Marqués de Valdecilla», Santander, Spain

Summary. We have studied 41 meningiomas classified histologically as benign, atypical or anaplastic. There were 26 females and 15 males and the mean age was 53 years. 36 tumours were supratentorial, 4 infratentorial and one spinal. Flow cytometry was performed on paraffin-embedded tissue using a selective staining technique for DNA. The ploidy index of DNA and percentage of cells in the S and G2/M phases were calculated. Results were correlated with clinical, histological and immunohistological data. 16/41 tumours were found to be diploid, 17/41 aneuploid and 8/41 could not be analysed. Significant correlations were found between aneuploid tumours and some qualitative features such as recurrence, pleomorphism, high cellular density, mitotic activity and brain and soft tissue infiltration. A high proliferative index appeared to be associated with clinical aggressiveness. No particular correlation between the expression of cytokeratin and epithelial membrane antigen markers and flow cytometry was found. Our results suggest that DNA flow cytometry in meningiomas may be of value in predicting the behaviour of these neoplasms and confirm that epithelial pattern in meningiomas is not linked to increased anaplasia or poor prognosis.

Key words: Meningiomas, DNA, Immunohistochemistry, Flow cytometry.

Introduction

Meningiomas are neoplasms which do not usually present diagnostic difficulties. Malignant meningiomas are rare (Russell and Rubinstein, 1989). However, recurrences, despite total excision and a benign histological appearance, are frequent (Crone, 1988). Histological criteria of anaplasia such as high cellularity, increased number of mitosis, cellular atypia, necrosis and cellular pleomorphism have been used to classify meningiomas into different groups according to their potential aggressiveness (Zülch, 1979; Alvarez et al., 1987). However, accurate prediction of poor prognosis or recurrence on the basis of histological parameters may be impossible (Gullotta and Wüllenweber, 1968; Rusell and Rubinstein, 1989).

Immunohistological expression of epithelial markers in some meningiomas has been found, and has been linked to increased anaplasia and to poor prognosis (Kepes, 1986). Some authors (Theaker et al., 1986; Terpe et al., 1988; Cruz-Sánchez et al., 1990) have related epithelial differentiation to cytogenesis of meningiomas.

Flow cytometry is a cytological assay which gives information on the cellular DNA content and on the proliferative activity of tumour cells (Crone et al., 1988). This technique has been applied to meningiomas (Hoshino et al., 1978; Ahyai et al., 1983) and correlation between flow cytometry results and clinical and pathological data has been reported by several researchers (Hoshino et al., 1976; Ironside et al., 1987; Crone et al., 1988). Correlation with immunohistological findings however has not been done. In the present study, results of flow cytometry in a series of 41 meningiomas were correlated with clinical, histological and immunohistological data in an attempt to predict the behaviour of these neoplasms.

Materials and methods

Forty-one meningiomas, from 26 females and 15 males, were available for study. The mean age of the patients was 53.5 years with a range between 21 and 75 years. Thirty-six tumours were supratentorial, 4 infratentorial and 1 was located in the spinal cord. Formalin-fixed, paraffin-embedded tissue was used for histological, immunohistological and flow cytometry analysis. Seven µm thick sections were stained with haematoxylin and eosin (H&E) and further sections had

Offprint requests to: Dr. F.F. Cruz-Sánchez, Banco de Tejidos Neurológicos, Servicio de Neurología, Hospital Clínico y Provincial, C/ Villarroel 170, 08036 Barcelona, Spain

a panel of monoclonal antibodies applied to them: the avidin-biotin peroxidase complex (ABC) method was applied to detect epithelial membrane antigen (EMA-Dako), cytokeratins (pancytokeratin [Boehringer], Cam 5.2. [Becton & Dickinson]), LP34 (Dako) and vimentin (Dako). Double immunostaining (for vimentin and pancytokeratin) was also performed in some selected cases, using diaminobenzidine (for vimentin) and alkaline phosphatase anti-alkaline phosphatase (for pancytokeratin).

Meningotheliomatous, fibroblastic transitional, angioblastic and secretory meningiomas were all included in the study group. Patients had a mean follow-up of 7.7 years. Fourteen tumours recurred over a mean of 2.8 years (range 1 to 6 years) and 3 patients died within 2 years (range 1 to 6 years) and 3 patients died within 2 years of the first operation.

Table 1 summarizes the clinical data in relation to the histological types of the tumours.

Table 1. Meningiomas. Clinical features in 41 cases. Summary of clinical data and histological type correlation with recurrency and follow-up.

CASE	AGE	SEX	LOCATION	HISTOL. TYPE	RECURRED AT (YEARS)	FOLLOW-UP (YEARS)
1	60	M	ST	MENINGOTHELIOMAT.	-	6
2*	30	M	ST	"	-	12
3	67	F	ST	"	-	13
4	21	M	IT	"		9
5	27	M	ST	"		11
6	50	M	ST	"		9
7	63	F	ST	"		11
8	66	M	ST	"		8
9	60	F	ST	"		10
10	45	M	IT	"		10
11	37	F	ST	"	-	10
12	62	M	ST	"	-	12
13*	63	F	ST	"	3	4
14	68	F	ST	"	4	8
15	63	F	ST	"	6	9
16*	50	F	ST	"	1	10
17*	62	M	ST	"	2	5
18	37	F	ST	FIBROBL.	-	8
19	37	M	SC	"	-	8
20	61	F	ST	"	-	9
21	73	F	IT	"	-	10
22	46	F	ST	TRANST.	-	9
23	53	M	ST	"	-	8
24*	71	F	ST	ANGIOBL.	2	8
25*	51	M	ST	"	2	12
26	70	F	ST	SECRETORY	-	3
27	50	M	ST	"	-	3
28*	70	F	ST	"	2	4
29	67	F	ST	"	3	3
30	30	M	ST	ANAPLASTIC	3	8
31	63	F	ST	"	1	1.5 (1)
32	68	F	ST	"	2	2 (1)
33	65	F	ST	"	2	2 (1)
34*	50	F	ST	ANGIOBL.	-	6
35	48	F	ST	FIBROBL.	-	6
36*	76	F	IT	"	-	5
37	**66**	**F**	**ST**	**TRANST.**	**-**	**8**
38	29	F	ST	FIBROBL.	-	8
39	34	F	ST	MENINGOTHELIOMAT.	-	8
40	38	M	ST	FIBROBL.	-	8
41*	40	F	ST	MENINGOTHELIOMAT.	-	4

M= male; F= female; ST= supratentorial; IT= infratentorial; SC= spinal cord; Fibrobl.= fibroblastic; Transt.= transitional; Angiobl.= angioblastic; Meningotheliomat.= meningotheliomatous; *= atypical meningioma; (1)= died at this time.

Flow cytometry

Suspensions of single nuclei were prepared from 50 μm sections of paraffin-embedded tissue according to Headley et al. (1983). Each sample was then treated with a solution of ribonuclease A and propidium iodide (both from Sigma, St. Louis, MO) according to Vindelov et al. (1982).

Flow cytometry was performed with an EPICS Profile-II (Counter Electronics, Inc. USA). At least 10,000 nuclei from each sample were analysed, with a mean number of 72,000. Normal lymph nodes as controls for the DNA index were processed in the same way as the tumours.

The mean coefficient of variation (CV) of all histograms was 5.7 and when a histogram had a CV>10 it was excluded.

Diploid tumours were those which demonstrated a simple or a multiple cell population with a different DNA index from diploid controls. Cell cycle analysis was performed using a programme for cytology (Coulter Electronics, USA). The number of cells in each phase was calculated and the results were expressed as a percentage of the whole sample. The proliferative index of each tumour was calculated by adding together the number of cells in the S -and G2/M- phases. Statistical analysis was performed using the SPSS programme (SPSS Inc.).

Results

Flow cytometry

DNA was measured in 33 meningiomas. Eight cases could not be analysed due to excessive debris or insufficient nuclei.

Sixteen meningiomas had diploid DNA content (Fig. 1) while 17 had aneuploid DNA index of 0.7 with a range of 0.5 to 0.8 (considered as hypoploid) (Fig. 2). The remaining 7 cases had a DNA index of 1.5 with a range of 1.3 to 2.2 and were considered to be hyperploid.

In hyperploid meningiomas, the percentage of cells in S-phase and the proliferative index could not be estimated because of overlap of cell populations (Fig. 3).

Table 2 shows the DNA index, the percentage of cells in S-phase and the proliferative index of each case.

Histological findings

The 41 tumours included 19 meningothelial, 8 fibroblastic, 3 transitional, 3 angioblastic, 4 secretory and 4 anaplastic meningiomas. Tumours were classified as benign, atypical or malignant, according to Alvarez et al. (1987). Atypical meningiomas (cases 2, 13, 16, 17, 24, 25, 28, 34, 36 and 41) showed a high mitotic rate and at least one of the other following histological features: high cellularity; atypical mitosis; necrosis or

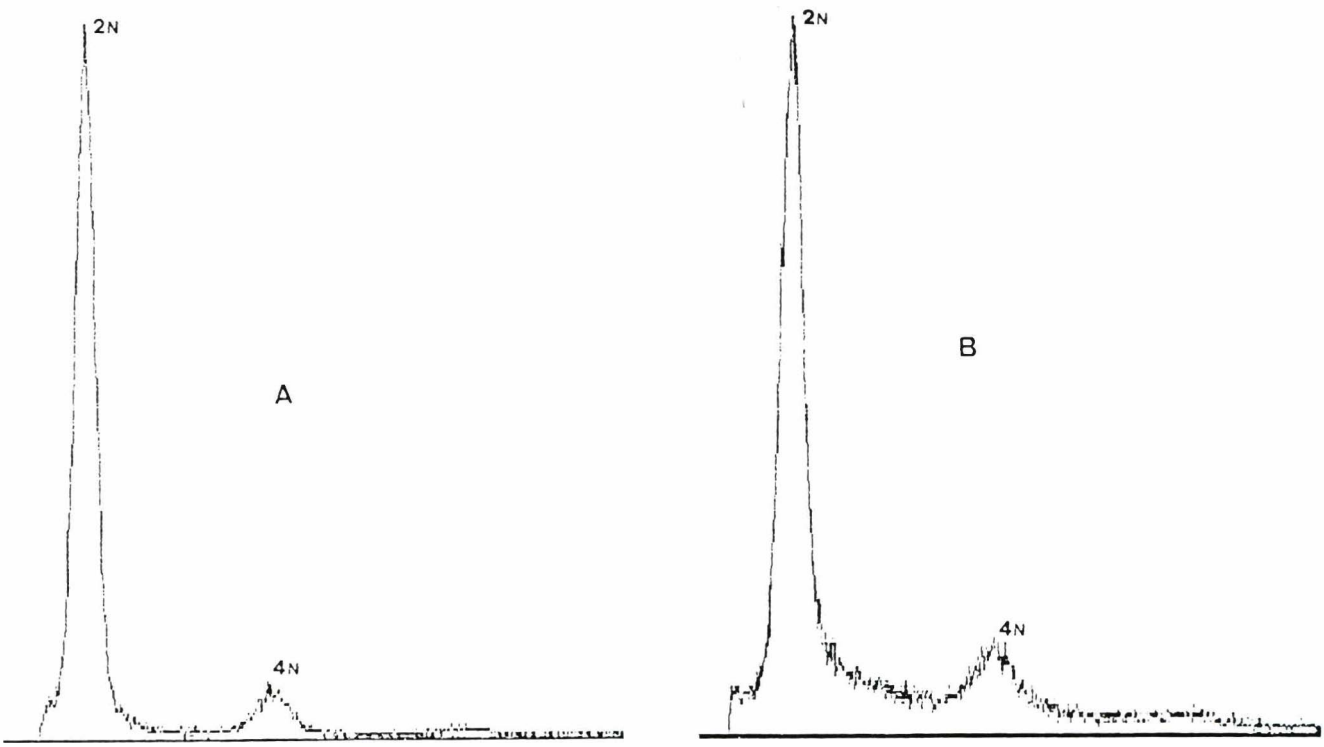

Fig. 1. Flow cytometry plot showing a diploid cell population in the first peak on the left (A and B) [2N]), corresponding to Go/G1 phase and a second peak corresponding to G2/M phase (4N). Note the difference in the value of the G2/M [4N] in B compared to A (compared with a normal lymphnode cell population).

pleomorphism. All of these features were seen in malignant meningiomas which also showed poor differentiation. Invasion of surrounding tissue was observed in 15 cases. Infiltration of brain was seen in 7 cases (cases 9, 11, 13, 17, 31, 32, 33), and bone and soft tissue infiltration was found in cases 31, 32 and 33.

Table 3 summarizes some histological characteristics of 41 meningiomas.

Immunohistological findings

Thirty-six tumours showed positivity for EMA. The staining was both cytoplasmic and membrane-bound. Few psammoma bodies were positive but some hyaline bodies showed strong EMA positivity. Twenty-five meningiomas were also positive with at least one of the 3 cytokeratin antibodies (4 cases were positive for LP34, 19 cases for Cam 5.2 and 25 cases for pancytokeratin). Both patterns of positivity, cytoplasmic and around psammoma and hyaline bodies, were observed. Co-expression between EMA and cytokeratin antibodies was also found in most cases.

Thirty cases showed diffuse positivity for vimentin with patchy accentuation. Some whorls and some cells arranged in fascicular bands were strongly vimentin positive. Hyaline and psammoma bodies and surrounding cells were negative for vimentin.

Using double-staining for vimentin and cytokeratin (pancytokeratin) 2 different patterns of staining were observed in some cases (Fig. 4). Co-expression of EMA and vimentin was found in some areas. These showed a homogeneous cytoplasmic pattern of positivity for both antibodies.

Correlation of data

5/17 aneuploid meningiomas were from patients aged between 21 and 50 years. 7/12 cases with a high proliferative index value were from patients between 21 and 50 years of age.

Most of the 11/13 cases which recurred were aneuploid; 5 hypoploid and 6 hyperploid. Five tumours (2, 10, 11, 12 and 25) with an aneuploid index had not recurred after a mean follow-up of 10 years. Most patients with recurrences were older than 50 years. Most aneuploid meningiomas were meningothelial (8 cases) as were 3/4 anaplastic meningiomas which had an aneuploid index (cases 31, 32 and 33). The remaining tumour (case 30) featured a combination of secretory and meningothelial patterns. Most atypical meningiomas

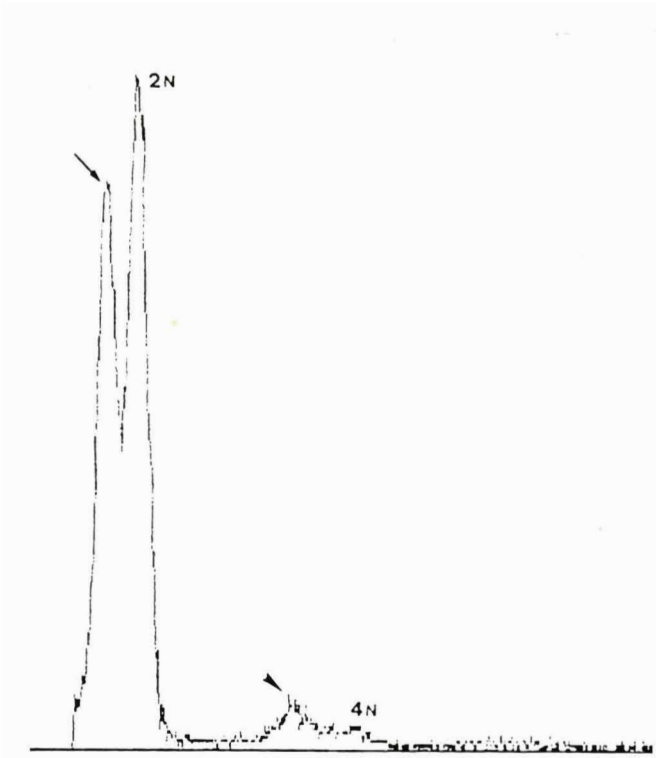

Fig. 2. Flow cytometry plot showing an aneuploid cell population (hypoploid) (arrow) and a second peak (2N) which corresponds to a diploid population. Note the corresponding G2/M phases in both peaks (arrowhead [from the aneuploid population and 4N from the diploid population]) (compared with a normal lymphnode cell population).

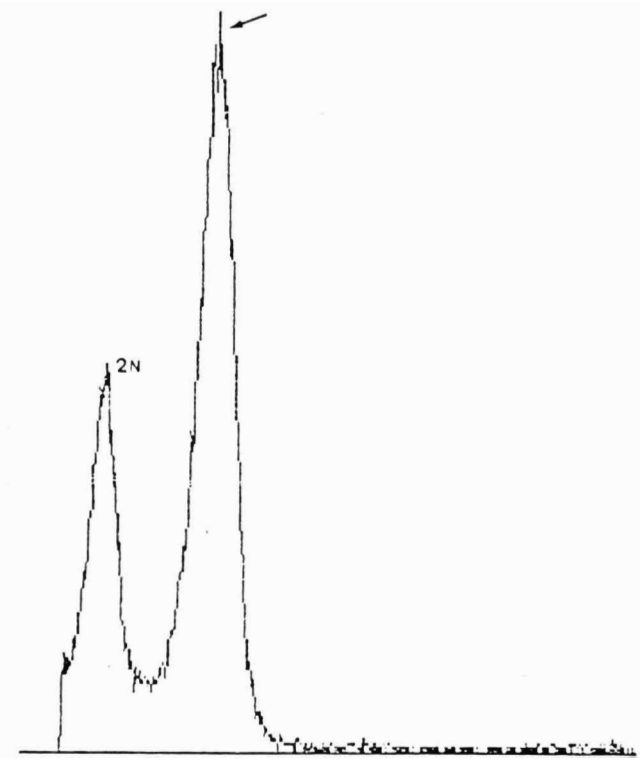

Fig. 3. Flow cytometry plot showing a diploid cell population (2N) and an aneuploid (hyperploid) cell population (arrowhead). G2/M phase are overlapping and cannot be distinguished (compared with a normal lymphnode cell population).

were aneuploid; 4 benign hyperploid and 2 hypoploid.

An aneuploid index correlated with necrosis in 4 cases and the mitotic index in 12 cases; 7 had a hyperploid, and 5 a hypoploid index. Case 30 had a diploid index with a proliferative index of 21.4 and histologically showed necrosis, mitotic activity, and high cellular density. Mononuclear cells infiltrating tumours were found in 22 cases; 12 of these were aneuploid and 5 diploid meningiomas, with a mean proliferative index of 26.36 (cases 6, 21, 22, 27 and 30).

Twelve cases showed high cellularity and most of them were aneuploid.

Table 4 demonstrates the significance values in relation to qualitative data which were obtained with contingency tables (chi-square) and with the Mann-Whitney rank sum test (SPSS programme from SPSS Inc.).

Table 2. Summary of flow cytometry results in 33 meningiomas. The DNA index is shown (DI) as well as the percentage of cells in S-phase and the proliferative index (PI) obtained by the sum of the S-phase plus the percentage of cells in the phase G2/M.

CASES	DI	S-PHASE	S- + G2/M-PHASES
1	1	4	6.5
2	1.3	25.6	29
3	0.9	1.8	12.7
4	0.9	2.9	3.8
5	1	3.5	9.6
6	1	21.1	27.2
7	1	4.9	5.8
8	1	7.9	9.9
9	1	11.5	19.8
10	0.7	7.9	11.5
11	0.8	11	22.5
12	0.8	6	15.1
13	1	3.7	7.4
14	0.8	17.2	21.8
15	0.7	20.7	21.9
16	0.5	22.6	25.2
17	1.5	-	-
18	0.8	11	12.5
19	1	7.2	10.4
20	1	4.9	7.1
21	1	7	16.9
22	1	7.5	14.2
23	0.8	3.8	8.3
24	0.6	3.8	10.5
25	1.6	-	-
26	1	5.4	8.7
27	1	15	22.1
28	1.8	-	-
29	2.2	-	-
30	0.9	12.3	21.4
31	1.5	-	-
32	1.3	-	-
33	0.8	7.1	8.8

Four tumours were positive for LP34 (10, 11, 18 and 40). Three of these tumours were studied by flow cytometry (cases 10, 11 and 18) and had a hypoploid index. However, only one of them (case 11) showed a high value of proliferative index (22.5). These 3 tumours had not recurred after a mean follow-up of 9.3 years. The presence of other epithelial antigens did not correlate with flow cytometry results either. No correlation between epithelial differentiation and recurrence or poor prognostic features was observed in any of the 41 meningiomas.

Discussion

Fresh or frozen tissues have been used in most flow cytometry studies of meningiomas (Frederiksen et al., 1979; Hoshino et al., 1978; Ahyai et al., 1983; Ironside et al., 1987; Crone et al., 1988). Hedley et al. (1983)

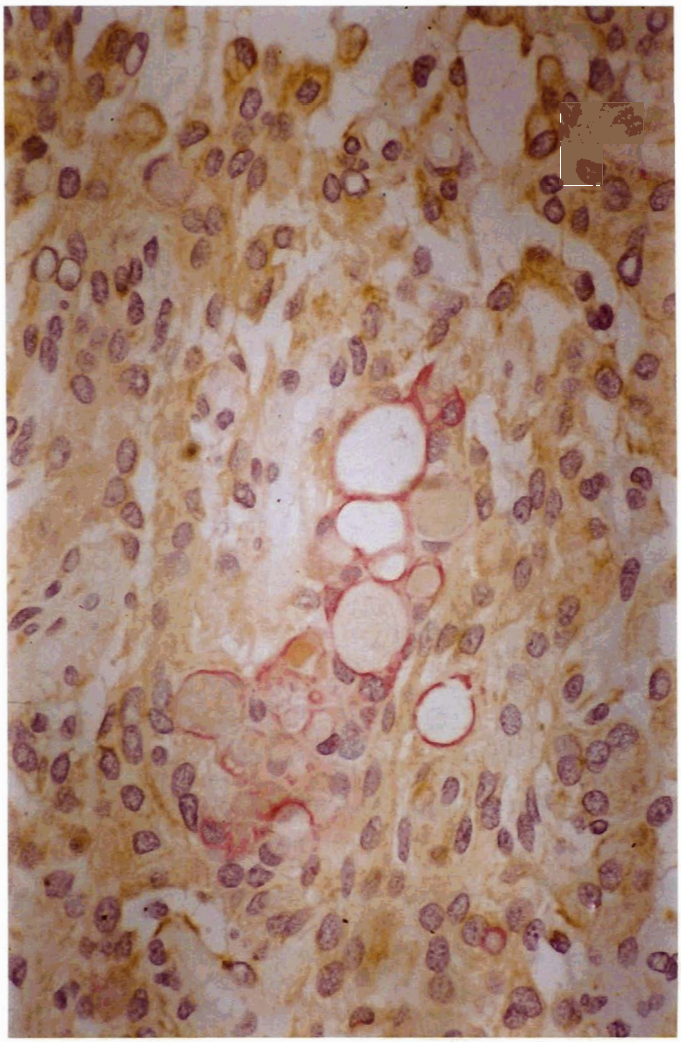

Fig. 4. Double immunostaining for vimentin (brown) and pancytokeratin (red). Note the rimmed pattern of positivity for cytokeratin. x 200

Table 3. Summary of the histological characteristics in 41 meningiomas and details anaplastic features.

CASES	CELLULAR DENSITY A	NUCLEAR PLEOMORPHISM B	MITOSIS C	NECROSIS D	INVASION E	MONONUCLEAR CELL INFILTRATE F
1	+					
2	+++		++			
3	++		-			
4	++					
5	++					
6	+++					++
7	++					-
8	++					+
9	++				+	-
10	++				-	++
11	++		+	-	+	++
12	++		-	-	-	-
13	++		++	+	+	++
14	+++			-		+++
15	+++			-		+
16	+++	-	++	-	-	++
17	++	+	++		+	++
18	+++					-
19	++					-
20	++					-
21	++					++
22	++					+
23	++	-	-			-
24	+++	++	+			++
25	++	++	+			+
26	++	-	-			-
27	++	+	-		-	++
28	+++	++	+		-	++
29	++	++	+		-	++
30	++	++	+	+++	-	+++
31	+++	+++	++	+++	+++	+++
32	+++	++	++	++	+++	+++
33	++	+	++	++	+++	+
34	++	++	+	-	-	-
35	++	-	-	-	-	-
36	++	++	++	-		-
37	++	-	-	-		-
38	++					
39	++					
40	+++	-	-			-
41	+++	++	++			++

A: += low, ++= moderate, +++= marked; B and F: -= absent, += mild/scanty/minimal, ++= moderate, +++= marked; C: -= absent, += an occasional mitose x high power field (HPF) (x400), ++= 1 mitosis in every other HPF, +++= 1 or more mitosis/HPF; D: -= absent, += mild, ++= moderate, +++= marked; E: invasion of neural tissue/bone/pericranial soft tissue, -= absent, += minimal, ++= moderate, +++= marked.

proposed a method to analyse the cellular DNA content of paraffin-embedded pathological material. This method is suitable for retrospective studies of tumours, such as meningiomas, with a long follow-up period. Correlation between flow cytometry and clinico-pathological data from meningiomas involving a long follow-up period, have not been reported up to now. Previous reports have shown that most meningiomas are diploid (Frederiksen et al., 1979; Ahyai et al., 1983). However, only a few papers have presented an analysis of the cell-cycle phase and proliferative index in meningiomas. Ironside et al. (1987) demonstrated that clinically aggressive meningiomas were aneuploid with high values for S and G2/M phases. Crone et al. (1988) reported an association between high proliferative index values and tumours featuring brain invasion, associated

Table 4. Significance values to qualitative data obtained with contingency tables (chi-square) and the Mann Whitney rank sum test (p).

FEATURES	DIPLOID/ANEUPLOID		HYPERPLOYD/HYPOPLOID	
	X2	P	X2	P
Sex	0.858	0.865	0.245	0.433
Recurrence	0.007	0.003 *	0.317	0.168
Histological type	0.623	-	0.202	-
Atypical meningiomas	0.330	0.187	0.689	0.060
Anaplastic meningiomas	0.639	0.368	0.732	0.408
Density	0.025	0.005 *	0.839	0.825
Pleomorphism	0.093	0.050 *	0.076	0.036
Mitosis	0.016	0.008 *	0.083	0.083
Necrosis	0.639	0.368	0.732	0.408
Invasion	0.093	0.050 *	0.433	0.245
Mononuclear cells Infiltrating tumors	0.223	0.130	0.864	0.751

*: Significant correlation.

oedema and recurrence. In our series hyperploid meningiomas were more strongly associated with recurrence. Most of these tumours showed an increased number of mitosis, cellular atypia, brain invasion, necrosis and/or cellular pleomorphism. In the remaining cases the correlation between DNA index and recurrence of histological features of aggressiveness revealed some discrepancies. Nevertheless, the most significant correlation was found with values of proliferative index. High proliferative index values were found more commonly when the patient's age was less than 50 years. Clinically, aggressiveness, recurrence and malignancy in meningiomas from a young population have been reported (Russell and Rubinstein, 1989). Most of these tumours showed high cellularity and/or increased number of mitosis. All of these features indicate that cellular proliferation in meningiomas may be increased in young people.

Few meaningful correlations were found between the results of flow cytometry and the histological subtypes. Angioblastic and anaplastic meningiomas were aneuploid or showed high proliferative index values and most atypical meningiomas which recurred had aneuploid indexes or high proliferative index values. Ironside et al. (1987) found similar features in relation to angioblastic and anaplastic meningiomas. In the present series, 3/4 anaplastic meningiomas demonstrated malignant behaviour and both angioblastic meningiomas analysed by flow cytometry recurred 2 years after the original operation. Histological features which often correlated with aneuploid index or high proliferative index values were mitotic activity, surrounding tissue invasion and numerous mononuclear cells infiltrating the tumours. However, some of these features were not statistically significant in relation to ploidy, which could be due to the small number of cases. Ironside et al. (1987) found that meningiomas with mitotic activity had G2/M-phase values greater than 10%, but equally high values were found in other neoplasms in which no mitosis were found. Crone et al. (1988) showed a significant correlation between brain and soft tissue invasion and aneuploid index or high cellular proliferation. Lastly, Rossi et al. (1988) found that the number of macrophages and T and CD8 lymphocytes in meningiomas were related to atypical histological features. Malignant and recurrent meningiomas from the present series showed heavier mononuclear cell infiltrates.

In our cases, the expression of epithelial markers did not correlate with the degree of anaplasia or aggressiveness. Follow-up also showed that epithelial differentiation did not correlate with poor prognosis. These results are in agreement with those of other authors (Theaker et al., 1986; Terpe and Anders, 1989; Cruz-Sánchez et al., 1990). Recently, Cruz-Sánchez et al. (1992) demonstrated that the expression of epithelial markers in meningiomas is common. These authors suggested that mesenchymal and epithelial elements are present in meningiomas and operate simultaneously in the histogenesis of these tumours and also that the epithelial pattern in meningiomas is not linked to aggressiveness, increased anaplasia or poor prognsosis. In the present study, flow cytometry results did not show particular correlation with the expression of epithelial markers either. Epithelial antigens may be found in aneuploid and hyperploid tumours, and tumours with high and low proliferative index values. These results support earlier histological and immunohistological findings.

Our finding support previous suggestions that flow cytometry in meningiomas may have a predicitive value in relation to the clinical behaviour of these tumour (Ironside et al., 1987). The association between aneuploidy and poor clinical outcome in meningiomas is not always present and the proliferative index appears to be more constantly associated with clinical aggressiveness. However, immunohistological studies correlating present data with proliferative indexes in fresh material could lead to more definite conclusions. Further studies in a larger series of patients (including long-term follow-up) are also necessary.

Acknowledgements. We would like to thank Mr. A. Pichardo, M. Mencia (supported by CIRIT) and Ms. M.J. Iranzo from the Neurological tissue bank for her excellent technical assitance. We thank Dr. Carey MP for his very helpful advice in the preparation of the manuscript. R. Miquel was supported by DGICYT, and this work was partially financiated by DGICYT PM 90/0060 and FISss 92/0754. We are thankful to Atom S.A. for the financial colour picture impression.

References

Ahyai A., Zimmerman A. and Spaar F.W. (1983). Flow fluorescence

cytometry of deoxyribonucleic acid in meningiomas: studies on surgically removed tumour specimens compared with their cells in primary tissue cultures. Surg. Neurol. 20, 194-205.

Alvarez F., Roda M.S., Romero M.P., Morales C., Sarmiento M.A. and Blázquez M.G. (1987). Malignant and atypical meningiomas: a reppraisal of clinical, histological and computed tomographic features. Neurosurgery 20, 688-694.

Crone K.R., Cholla V.R., Kute T.E., Moody D.M. and Kelly D.L. (1988). Relationship between flow cytometric features and clinical behaviour of meningiomas. Neurosurgery 23, 720-724.

Cruz-Sánchez F.F., Rossi M.L., Marcel G.B., Ferrer I. and Rodríguez-Prados S. (1990). Epithelial markers in «intradural» tumours. Neuropathol. Appl. Neurobiol. 16, 271 (abstract).

Cruz-Sánchez F.F., Rossi M.L., García Bach M., Rodríguez-Prados S., Ferrer I. and Coakham H.B. (1992). Epithelial differentiation in gliomas, meningiomas and choroid plexus papillomas cancer. Virchows Archiv. (B) 62, 25-34.

Frederiksen P., Reske-Nielsen E. and Buchel P. (1979). DNA content of meningiomas. Acta Neuropathol. (Berlin) 46, 65-68.

Gullota F. and Wullenweber R. (1968). Zur Frage den malignen Emtartung bei Menengeom und Meningeom-recidiv. Acta Neurochir. (Wien) 18, 15-27.

Hedley D.W., Friedlander M.L. and Taylor I.W. (1983). Method for analysis of cellular DNA content of paraffin-embedded pathological material using flow cytometry. J. Histochem. Cytochem. 31, 1333-1338.

Hoshino T., Nomura K., Gray J.W. and Wilson C.B. (1976). Flow microfluorometric studies of brain and brain tumours. J. Neuropathol. Exp. Neurol. 35, 363.

Hoshino T., Nomura K., Wilson C.B., Krebel K.D. and Gray J.W. (1978). The distribution of nuclear DNA from human brain tumour cells. J. Neurosurg. 49, 13-21.

Ironside J.W., Battersby R.D.E., Lawry J., Loomes R.S., Day C.A. and Timperley W.R. (1987). DNA in meningiomas tissues and explant cell cultures. A flow cytometric study with clinicopathological correlates. J. Neurosurg. 66, 588-594.

Kepes J.J. (1986). The histopathology of meningiomas. A reflection of the origins and expected behaviour. J. Neuropathol. Exp. Neurol. 45, 95-107.

Rossi M.L., Cruz-Sánchez F.F., Hughes J.T., Esiri M.M. and Coakham H.B. (1988). Immunohistochemical study of the cellular immune response in meningiomas. J. Clin. Pathol. 41, 314-319.

Russell D.S. and Rubinstein L.J. (1989). Tumours of the meninges and related tissues. In: Pathology of tumours of the nervous system. 5 th Ed. Russell D.S. and Rubinstein L.J. (eds). Edward Arnold Publisher. London. pp 449-494.

Terpe H.J., Kasper M., Martin H. and Lehman J. (1988). Nachweis von Zytokeratin in Zellen der Arachoidea und in Meningiomen Zentralbl. Allg. Pathol. 134, 259-264.

Terpe H.J. and Anders M. (1989). The immunohistochemical reactivity of an anti-epithelial monoclonal antibody (b-12) in meningiomas. Acta Histochem. 87, 77-79.

Theaker S.M., Gatter K.C., Esiri M.M. and Fleming K.A. (1986). Epithelial membrane antigen and cytokeratin expression by meningiomas: an immunohistological study. J. Clin. Pathol. 39, 435-439.

Vindelov L.L., Christensen I.B.J., Keiding N., Spang-Thomsen M. and Nissen N.I. (1982). Long term storage os samples for flow cytometry DNA analysis. Cytometry 317-322.

Zulch K.S. (1979). International histological classification of tumours No. 21. World Health Organization, Geneva.

Accepted July 10, 1992