Cerebrospinal fluid tumour markers in patients treated for meningeal malignancy

Anton P van Zanten, Albert Twijnstra, Bram W Ongerboer de Visser, Peter van Heerde, Augustinus A M Hart, William J Nooyen

Abstract

The results of cerebrospinal fluid (CSF) biochemical markers were compared with conventional CSF cytology in patients treated for leptomeningeal metastases from extra cranial malignancies. For lumbar CSF, before treatment, no statistically significant difference of the probabilities of being positive was found between CSF cytology and a classification by linear discriminant analysis, based on patient's age, of β -glucuronidase and β_2 -microglobulin. During treatment, classification by linear discriminant analysis was found more often positive than cytology. Possible mechanisms for this difference are discussed. For ventricular CSF a correlation was found between CSF cytology and β -glucuronidase for solid tumours, and between CSF cytology and β_2 -microglobulin for haematological malignancies. Reference values for ventricular protein, CEA β -glucuronidase and β_2 -microglobulin were obtained for cytological negative samples.

The diagnosis of leptomeningeal metastasis is usually established by the demonstration of tumour cells in the cerebrospinal fluid (CSF). Occasionally, the failure to demonstrate tumour cells in the CSF of patients with the clinical signs and symptoms of leptomeningeal metastasis constitutes a difficult diagnostic problem.^{1,2} Diagnostic tools, such as the determination of CSF tumour markers, offering the possibility of an early detection of leptomeningeal metastasis, have been the subject of several studies.³⁻¹⁴

As intrathecal chemotherapy may be effective in countering meningeal spread of some tumours, markers are also needed during follow up studies, particularly because clinical evaluation may be difficult when irreversible damage to the nervous system has happened.

In previous studies³⁻⁷ we estimated the test characteristics sensitivity and specificity for the detection of meningeal malignancy of several biochemical markers in lumbar cerebrospinal fluid. We found that β -glucuronidase and β_2 -microglobulin were the most useful markers for meningeal spread from solid tumours and haematological malignancies, respectively.

With the introduction of the use of the Ommaya device in the treatment of leptomeningeal metastasis, the ventricular CSF became

readily available for analysis of the biochemical markers. In this study we analysed the results of the markers carcinoembryonic antigen (CEA), β -glucuronidase, lactate dehydrogenase (LDH), β_2 -microglobulin and of routine glucose and total protein, during therapy in relation to the findings of lumbar and ventricular CSF cytology.

Methods

Twenty five patients were referred to the Netherlands Cancer Institute for treatment. The primary site of malignancy, the follow up period and the number of CSF samples for each patient are given in table 1. The diagnosis of leptomeningeal metastasis was confirmed in all cases by a positive CSF cytology.

Three patients were already being treated when this study started. All patients were treated with intraventricular methotrexate (MTX), six patients in combination with cranial radiotherapy. No steroids or other symptomatic treatments were used. None of the patients is still being treated.

Ventricular and lumbar punctures were carried out as part of the diagnostic and treatment procedures but not for the measurements of the markers alone. To obtain ventricular CSF, the Ommaya reservoir was inserted with a 23 gauge butterfly needle. Three ml of CSF, which had passed through the reservoir and tubing was withdrawn and discarded. An additional 7 ml was then removed from the ventricle for the purpose of sampling. When clinically necessary, a total of 10 ml CSF was also removed by lumbar puncture one hour after the sampling from the Ommaya reservoir.

For cytological examination, slides were prepared by the cytocentrifuge technique (Shandon Elliot cytopspin). The slides were airdried, fixed in methanol and routinely stained with Giemsa.

After centrifuging the samples, glucose, protein and LDH were determined immediately. Protein was measured using an adaptation on the ACA of the trichloroacetic acid procedure described by Meulemans.¹⁵ Glucose was measured enzymatically with an oxygen electrode (Beckman Instruments, Fullerton, USA). LDH was determined with the Automated Clinical Analyzer test method, calibrated to give values comparable with the method of the German Society for Clinical Chemistry.⁴ Additional samples were coded

Municipal Hospital,
Slotervaart,
Amsterdam
Department of
Clinical Chemistry
A P van Zanten
Department of
Neurology
B W O de Visser*
Netherlands Cancer
Institute, Amsterdam
Clinical Division*
A Twijnstra
Department of
Pathology
P van Heerde
Department of
Radiotherapy
A A M Hart
Department of
Clinical Chemistry
W J Nooyen

Correspondence to:
Dr van Zanten, Department
of Clinical Chemistry,
Municipal Hospital
Slotervaart, Louwesweg 6,
1066 EC Amsterdam, The
Netherlands.

Received 10 July 1989 and in
revised form 27 March 1990.
Accepted 2 May 1990

Table 1 Patients treated for leptomeningeal spread from solid tumours and haematological malignancies

Patient	Sex/Age	Tumour	Follow Up	Number of CSF Samples					
				Lumbar			Ventricular		
				Cyt	+	-	+	-	
1	F/22	ALL	203	—	—	—	1	5	
2	M/52	M Hodgkin	47	1	1	—	3	4	
3	M/61	NHL	36	—	—	—	4	1	
4	M/49	NHL	50	1	1	—	6	1	
5	F/70	NHL	46	—	—	—	—	5	
6	M/51	NHL	11	2	—	—	3	—	
7	M/34	NHL	116	2	2	3	3	7	
8	M/69	NHL	32	1	1	6	6	2	
9	F/68	Breast	50	2	1	2	2	9	
10	F/43	Breast	212	2	3	2	2	7	
11	F/63	Breast	18	1	2	—	—	3	
12	F/69	Breast	463	2	—	—	11	10	
13	F/45	Breast	195	1	—	—	1	7	
14	F/50	Breast	973	—	2	4	4	23	
15	F/50	Breast	70	2	2	2	2	7	
16	F/59	Breast	151	3	3	3	3	11	
17	F/57	Breast	64	3	—	—	8	—	
18	F/32	Breast	187	2	—	—	4	4	
19	F/48	Breast	182	4	—	—	4	13	
20	F/62	Breast	63	2	5	—	—	11	
21	F/52	Breast	835	2	1	19	10	10	
22	M/54	SCLC	52	1	5	1	1	10	
23	F/45	Non-SCLC	133	2	—	—	6	8	
24	M/31	Melanoma	29	3	—	—	10	—	
25	F/48	Melanoma	22	1	—	—	4	1	
Total				40	29	107	159		

The follow up period is defined as the number of days between the submission of the first and last CSF sample. ALL indicates acute lymphatic leukemia; NHL, non-Hodgkin lymphoma; SCLC, small cell lung carcinoma.

and stored at -20°C . β -glucuronidase was measured with phenolphthalein glucuronide as substrate.³

β_2 -microglobulin was determined by radioimmunoassay with the Phadebas β_2 -Micro Test⁵ and CEA levels with a modification⁶ of the method described by Persijn and Korsten.¹⁶ Our laboratory reference values for lumbar CSF are: protein 0.14–0.51 g/l; glucose 2.5–4.2 mmol/l; LDH 0–26 U/l; β -glucuronidase 9–27 mU/l; β_2 -microglobulin (standardised to age) 0.65–2.20 mg/l; CEA $<4\text{ }\mu\text{g/l}$.

In a previous study¹⁷ stepwise linear discriminant analysis (LDA) was carried out to classify the lumbar CSF samples into two categories: leptomeningeal spread present or absent. The discriminant functions for this analysis were generated from observations on a total of 265 patients, with metastatic and non metastatic diseases. In this study, the patients were classified by evaluating the scores obtained by the discriminant score functions from the previous study and assigning the patient to the group whose discriminant score function had the maximum score, that is, leptomeningeal spread present or absent.

The ventricular samples from each patient were divided into two categories, positive and

Table 3 Comparison of the results of an allocation based on LDA of the combination of patients' age, β -glucuronidase and β_2 -microglobulin with cytology in lumbar CSF samples, obtained prior to treatment

		Allocation by LDA	
		Positive	Negative
C	Positive	15	2
Y			
T			
O	Negative	1	0
L			
O			
G			
Y			

negative cytology, on the basis of cytological analysis. Differences between categories were evaluated with the two tailed, paired Wilcoxon test.

Results

For 28 paired samples, obtained from 13 patients, a comparison could be made between lumbar and ventricular CSF, obtained at the same day. In 22 cases, the results of lumbar and ventricular cytology were similar (15 negative and seven positive). In five cases the lumbar cytology was positive, although ventricular cytology was negative. In only one case did a positive ventricular cytology coincide with a negative lumbar cytology. The difference between lumbar and ventricular CSF cytology was not statistically significant (McNemar $p = 0.22$).

In lumbar CSF values for protein, LDH, β -glucuronidase, β_2 -microglobulin, and CEA were higher than those measured in the ventricular CSF. In contrast, ventricular CSF showed the highest glucose values (table 2).

By an allocation procedure, based on LDA of the combination of patient's age and the marker values for β -glucuronidase and β_2 -microglobulin in lumbar CSF, it was possible to allocate the lumbar CSF samples in two categories, that is, meningeal spread present or absent. Table 3 presents the results for 18 samples obtained at diagnostic puncture. In 32 lumbar samples, obtained during follow up (table 4) a statistically significant difference was found (McNemar $P = 0.036$) between the probability to be positive by LDA classification and cytology. In three of the 12 samples, with positive markers and negative cytology, there was evidence of infectious complications. By omitting these samples from the statistical

Table 2 Comparison of assay results in lumbar and ventricular CSF obtained at the same day

Analysis	No	Lumbar CSF			Ventricular CSF		
		Mean	Median	(SD)	Mean	Median	(SD)
Protein (g/l)	26	2.32	1.70	(1.72)	0.66	0.49	(0.66)
Glucose (mmol/l)	26	3.56	3.60	(1.01)	4.00	3.80	(0.80)
LDH (U/l)	25	81.20	54.00	(65.0)	18.50	15.00	(18.00)
β -glucuronidase (mU/l)	22	80.86	46.50	(134.3)	19.27	14.50	(15.60)
β_2 -microglobulin (mg/l)	23	2.56	2.09	(1.28)	1.29	0.93	(0.81)
CEA ($\mu\text{g/l}$)	23	10.40	3.10	(26.5)	7.92	2.30	(25.61)

Table 4 Comparison of the results of an allocation based on LDA of the combination of patients' age, β -glucuronidase and β_2 -microglobulin with cytology in lumbar CSF samples, obtained during treatment

		Allocation by LDA	
		Positive	Negative
C	Positive	9	3
Y			
T			
O	Negative	12	8
L			
O			
G			
Y			

Table 5 Mean values for the various parameters in ventricular CSF of patients treated for leptomeningeal metastases

Patient no	Protein		Glucose		LDH		β -glucuronidase		β_2 -microglobulin		CEA	
	+	—	+	—	+	—	+	—	+	—	+	—
	g/l		mmol/l		U/l		mU/l		mg/l		μ g/l	
1	0.23	0.32	2.60	3.44	1.0	1.2	na	8.0	0.92	0.72	2.20	1.99
2	0.25	0.47	4.77	4.32	6.7	5.7	8.7	8.0	1.86	1.05	1.18	1.38
3	0.30	na	4.05	na	44.5	na	14.5	na	1.91	0.53	1.62	2.11
4	0.48	0.36	3.83	4.50	32.3	16.0	11.6	14.0	0.70	0.43	2.30	1.86
5	na	0.39	na	4.70	na	6.8	na	8.6	na	1.26	na	1.68
6	0.55	na	4.43	na	34.0	na	8.0	na	4.10	na	1.67	na
7	0.83	0.50	4.17	4.20	11.3	13.4	10.5	14.2	3.73	1.64	2.00	1.74
8	0.82	0.50	3.47	3.50	44.3	15.0	22.0	19.0	1.93	1.77	2.17	2.22
9	0.43	0.76	4.15	4.28	21.0	7.3	26.0	24.9	1.32	2.30	2.25	3.12
10	0.46	0.29	4.0	3.91	25.0	2.3	17.0	10.3	0.56	0.61	2.15	2.84
11	na	0.39	na	3.37	na	18.7	na	21.7	na	1.53	na	3.38
12	0.33	0.33	3.44	3.69	14.7	17.0	15.5	14.6	1.21	1.92	2.20	2.17
13	0.41	0.27	3.80	3.85	26.0	8.6	19.0	12.0	1.12	1.20	2.65	2.21
14	0.49	0.57	3.05	3.64	23.5	8.7	15.3	15.1	1.71	1.21	1.64	3.22
15	0.19	0.55	4.70	4.40	3.5	7.9	10.5	10.6	0.73	1.07	1.80	2.54
16	0.78	0.32	5.63	5.08	10.0	15.9	14.0	11.4	1.05	1.12	3.42	2.21
17	0.25	na	3.45	na	20.0	na	16.4	na	2.19	na	85.64	na
18	0.19	0.18	6.12	3.67	15.0	13.0	17.0	11.0	0.81	0.82	2.35	2.38
19	0.51	0.55	4.05	4.12	10.0	4.9	21.3	12.0	0.58	0.85	2.34	2.16
20	na	0.89	na	3.67	na	19.4	na	26.2	na	1.91	na	2.17
21	1.38	0.89	3.85	4.03	15.6	16.6	43.9	30.7	1.44	1.40	2.70	2.78
22	0.29	0.17	4.70	4.94	92.0	9.6	49.0	16.7	0.32	0.32	2.00	1.79
23	0.20	0.24	4.08	4.11	11.3	10.7	15.0	14.0	3.06	1.38	20.62	13.54
24	0.38	na	3.58	na	12.8	na	15.4	na	0.84	na	2.49	na
25	0.65	0.40	3.77	4.20	7.5	10.0	12.5	10.0	0.68	0.44	2.20	1.40

+ Indicates sample with positive cytology; —negative cytology
na no value available

evaluation, the significance of the difference between LDA classification and cytology decreased (McNemar $p = 0.15$).

The results of the determination of the marker values in ventricular CSF are presented in table 5. Considering only those patients with positive and negative cytology a significant difference between marker values in samples with positive and negative cytology (table 6) was only found for β -glucuronidase (Wilcoxon, $p < 0.01$) and β_2 -microglobulin (Wilcoxon, $0.02 < p \leq 0.05$) for solid tumours and haematological malignancies, respectively.

Reference values for ventricular CSF were estimated with Neumann's method.¹⁸ For this analysis all ventricular samples with a negative cytology were used. We found: protein 0.10–0.28 g/l; glucose 2.8–4.9 mmol/l; β -glucuronidase 5–17 U/l; β_2 -microglobulin 0.22–1.80 mg/l; CEA 1.0–2.8 μ g/l. Reference values for LDH could not be calculated with Neuman's method. This was due to the many identical results measured by the ACA. This was caused by the low LDH activities measured in ventricular CSF and an electronic

circuit in the ACA, which produces only results which are multiples of two, for example, 0, 2, 4, 6, etc U/l.

Discussion

In evaluating the diagnostic value of CSF tumour markers in patients treated for leptomeningeal metastasis, there are several serious problems that need to be confronted. These are connected with the site of CSF puncture, the effects of therapy on blood/CSF barrier and with estimating reference values, particularly in ventricular CSF.

During the investigations leading to the diagnosis of leptomeningeal metastasis, it is usually lumbar CSF which is submitted to the clinical and cytological laboratory. During therapy, and after the installation of an Ommaya device, it is ventricular CSF which is usually submitted.

The composition of CSF can differ considerably between different CSF sources, such as the Ommaya reservoir or the lumbar space, even in the absence of an extra dural block.¹⁹ Reference values should therefore be estimated for lumbar and for ventricular CSF. For lumbar CSF, the methods for obtaining our reference values are described elsewhere.^{3–6} For ventricular CSF, the methods used for lumbar CSF cannot be used, mainly because of problems obtaining ventricular CSF for reference purposes. As only ventricular CSF from patients treated for their leptomeningeal metastases is submitted to our laboratory, we estimated the ventricular reference values with Neuman's method¹⁸ applied to the ventricular samples with a negative cytology.

From the literature,²⁰ it is known that results from CSF protein are influenced by the therapy used for the treatment of leptomeningeal metastases. It was also found²¹ that the administration of intrathecal methotrexate and CNS irradiation both affect endothelial pinocytosis, resulting in an increased transendoth-

Table 6 Mean and median of the results of biochemical markers in ventricular CSF for patients with positive as well as negative cytology

Analysis	No of patients	Mean	Median (SD)	Mean	Median (SD)
Solid tumours					
Protein, g/l	13	0.49	0.43 (0.32)	0.42	0.33 (0.22)
Glucose, mmol/l	13	4.26	4.05 (0.85)	4.15	4.11 (0.45)
LDH, U/l	13	21.2	15.0 (22.4)	10.2	9.69 (4.45)
β -glucuronidase, mU/l	13	21.2	17.0 (11.9)	14.9	12.0 (6.20)
β_2 -microglobulin, μ g/l	13	1.12	1.05 (0.70)	1.13	1.12 (0.56)
CEA, μ g/l	13	3.72	2.25 (5.10)	3.26	2.38 (3.13)
Haematological malignancies					
Protein, g/l	5	0.52	0.48 (0.29)	0.43	0.47 (0.08)
Glucose, mmol/l	5	3.77	3.83 (0.83)	3.99	4.25 (0.49)
LDH, U/l	5	19.1	11.3 (18.4)	10.3	13.4 (6.48)
β -glucuronidase, mU/l	4	13.2	13.2 (5.99)	13.8	14.1 (4.50)
β_2 -microglobulin, μ g/l	6	1.84	1.88 (1.07)	1.02	0.88 (0.57)
CEA, μ g/l	6	1.91	2.08 (0.43)	1.88	1.92 (0.30)

+ Indicates samples with positive cytology; —indicates negative cytology. The significance of the difference between mean results in patients with a positive as well as a negative cytology was calculated with the two tailed, paired Wilcoxon's test.

elial protein transport. The reference values for CSF protein, estimated by us in the ventricular CSF of patients treated for leptomeningeal metastasis, are somewhat higher than the published ventricular protein values²² but do, however, agree with the proposals by Livrea *et al.*²¹ The reference values for ventricular glucose appeared reliable, compared with the observations by Fishman,²³ who stated that the glucose content of ventricular CSF is usually higher than that of lumbar CSF. The β_2 -microglobulin and β -glucuronidase content of ventricular CSF was lower than that of lumbar CSF.

In comparing our results with the reference procedure cytology, it is necessary to know the clinical value of this technique. At presentation this technique has a sensitivity of 59%,¹ but repeated puncture increased the sensitivity to 91%.²⁴ It has been argued that immunocytochemistry should be superior to conventional CSF cytology.²⁵ However, in a group of 20 consecutive patients treated for leptomeningeal metastasis, we found no statistically significant difference between cytology and immunocytochemistry²⁶ (McNemar: $p = 0.77$). Differences between the two techniques were invariably found in samples with a low cell count. They were always based on a single or a few positive cells.

In a previous article¹⁷ we described the classification of lumbar CSF samples in two categories, leptomeningeal metastasis present or absent, using the patient's age and the results of β -glucuronidase and β_2 -microglobulin. These CSF samples were from patients, not yet treated for their leptomeningeal metastases. Although care should be taken when applying a computer model where it has previously not been used, it was interesting to test the model on patients treated for their leptomeningeal metastases. As might be expected, the agreement between the classification by LDA and cytology, in patients not yet being treated, was good. In contrast a statistical significant difference was found between the two techniques in the lumbar samples of the patients during treatment for their leptomeningeal metastases. These differences may be caused by increased turnover in the CSF of serum proteins as a side effect of CNS irradiation or the administration of methotrexate. Otherwise it may be caused by infectious complications sometimes seen during the treatment of patients with leptomeningeal metastases.

For ventricular CSF we compared the test results of patients from whom samples with positive and negative cytology were submitted to the laboratory. In this way, the influence of three patients with only positive and three patients with only negative ventricular cytology was excluded from our evaluation. For ventricular CSF we found a statistically significant correlation between cytology and β -glucuronidase for solid tumours and between β_2 -microglobulin and cytology for haematological malignancies. For β_2 -microglobulin, our results in patients with haematological malignancies agree with those obtained by Storti *et al.*¹² It should be noted

that opinions vary about the clinical value of CSF β_2 -microglobulin.^{13,14}

In conclusion, when interpreting CSF tumour markers during therapy they should be compared with test results with a reference range estimated for a specific region of the neuraxis, and that bacterial or fungal infections in particular should be excluded from the diagnosis by careful bacteriological examination of the CSF. During therapy the measurements of CSF tumour markers are not superior to CSF cytology in samples with elevated cell counts. Perhaps CSF tumour markers are most useful in samples with low cell counts, although this subject merits further serious attention.

- 1 Glass JP, Melamed M, Chernik NL, Posner JB. Malignant cells in cerebrospinal fluid (CSF): The meaning of a positive CSF cytology. *Neurology* 1979;29:1369-75.
- 2 Ongerboer de Visser BW, Somers R, Nooyen WH, van Heerde P, Hart AAM, McVie JG. Intraventricular methotrexate therapy of leptomeningeal metastasis from breast carcinoma. *Neurology* 1983;33:1565-72.
- 3 van Zanten AP, Twijnstra A, van Benthem V, Hart AAM, Ongerboer de Visser BW. Cerebrospinal fluid β -glucuronidase activities in patients with central nervous system metastases. *Clin Chim Acta* 1985;147:127-34.
- 4 van Zanten AP, Twijnstra A, Hart AAM, Ongerboer de Visser BW. Cerebrospinal fluid lactate dehydrogenase activities in patients with central nervous system metastases. *Clin Chim Acta* 1986;161:259-68.
- 5 Twijnstra A, van Zanten AP, Nooyen WJ, Hart AAM, Ongerboer de Visser BW. Cerebrospinal fluid β_2 -microglobulin: a study in controls and patients with metastatic and non metastatic neurological diseases. *Eur J Cancer Clin Oncol* 1986;22:387-91.
- 6 Twijnstra A, Nooyen WJ, van Zanten AP, Hart AAM, Ongerboer de Visser BW. Cerebrospinal fluid carcinoembryonic antigen in patients with metastatic and non metastatic neurological diseases. *Arch Neurol* 1986;43:269-72.
- 7 van Zanten AP, Twijnstra A, Ongerboer de Visser BW. Routine investigations of the CSF with special reference to meningeal malignancy and infectious meningitis. *Acta Neurol Scand* 1988;77:210-14.
- 8 Schold SC, Wasserstrom WR, Fleisher M, Schwartz MK, Posner JP. Cerebrospinal fluid biochemical markers of central nervous system metastasis. *Ann Neurol* 1980;8:597-604.
- 9 Shuttleworth EC, Allen N. CSF β -glucuronidase assay in the diagnosis of neoplastic meningitis. *Arch Neurol* 1980;37:684-7.
- 10 Tallman RD, Kimbrough SM, O'Brien JF, Goellner JR, Yanagihara T. Assay for β -glucuronidase in cerebrospinal fluid: Usefulness for the detection of neoplastic meningitis. *Mayo Clin Proc* 1985;60:293-8.
- 11 Jacobi C, Reiber H, Felgenhauer K. The clinical relevance of locally produced carcinoembryonic antigen in cerebrospinal fluid. *J Neurol* 1986;233:358-61.
- 12 Storti S, Pagano L, Marra L, Teofilii BM, Ricerra GL, Bizzi B. Cerebrospinal fluid beta-2-microglobulin: a reliable index of leukaemic infiltration of central nervous system. *Scand J Haematol* 1986;37:301-5.
- 13 Nagelkerke AF, van Kamp GJ, Veerman AJP, de Waal FC. Unreliability of β_2 -microglobulin in early detection of central nervous system relapse in acute lymphoblastic leukemia. *Eur J Cancer Clin Oncol* 1985;21:659-63.
- 14 Pudek MR, Chan KW, Rogers PC, Teasdale JM. β_2 -microglobulin levels in cerebrospinal fluid of children with leukemia and lymphoma. *Clin Biochem* 1985;18:180-3.
- 15 Meulemans O. Determination of total protein in spinal fluid with sulphosalicylic acid and trichloroacetic acid. *Clin Chim Acta* 1960;5:757-61.
- 16 Persijn JP, Korsten CB. The development of a radio immunoassay for carcino-embryonic antigen with some applications. *J Clin Chem Clin Biochem* 1976;14:377-87.
- 17 van Zanten AP, Twijnstra A, Ongerboer de Visser BW, Hart AAM, Nooyen WJ. Tumormarkers in the cerebrospinal fluid of patients with central nervous system metastases from extracranial malignancies. *Clin Chim Acta* 1988;175:157-66.
- 18 Neuman GJ. The determination of normal ranges from routine laboratory data. *Clin Chem* 1968;14:979-88.
- 19 Murray JJ, Greco FA, Wolf SN, Hainsworth JD. Neoplastic meningitis marked variations of cerebrospinal fluid composition in the absence of extradural block. *Am J Med* 1983;75:289-94.
- 20 Duttera MJ, Bleyer WA, Pommeroy TC, Leventhal CM, Leventhal BG. Irradiation, methotrexate toxicity and the treatment of meningeal leukemia. *Lancet* 1973;ii:703-7.
- 21 Livrea P, Trojano M, Simone IL, *et al.* Acute changes in blood-CSF barrier perm selectivity to serum proteins

- after intrathecal methotrexate and CNS irradiation. *J Neurol* 1985;231:336-9.
- 22 Fishman RA. Composition of cerebrospinal fluid. *Cerebrospinal fluid in diseases of the nervous system*. Philadelphia: WB Saunders, 1980:206.
 - 23 Fishman RA. Composition of cerebrospinal fluid. *Cerebrospinal fluid in diseases of the nervous system*. Philadelphia: WB Saunders, 1980:211.
 - 24 Wasserstrom WR, Glass JP, Posner JP. Diagnosis and treatment of leptomeningeal metastasis from solid tumours: experience with 90 patients. *Cancer* 1982;49:759-72.
 - 25 Coakham HB, Garson JA, Brownell B, Kemshead JT. Diagnosis of Cerebral Neoplasms Using Monoclonal Antibodies. In: Clifford Rose F, Fields WS, eds. Proceedings of the Mansell Bequest Symposium on Advances in Neuro-Oncology. *Prog exp Tumor Res (Basel)* 1985;29: 57-77.
 - 26 Boogerd W, Vroom ThM, van Heerde P, Brutel de la Riviere G, Peterse JL, van der Sande JJ. CSF cytology versus immunocytochemistry in meningeal carcinomatosis. *J Neurol Neurosurg Psychiatry* 1988;51:142-5.