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Alpha-interferon responses in cerebrospinal fluid of patients with suspected meningitis

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SUMMARY Cerebrospinal fluid from 100 patients with clinically diagnosed meningitis was examined for α-interferon. In the laboratory four patient groups were identified: bacterial meningitis (n = 12), viral meningitis (n = 15), normal cerebrospinal fluid (n = 57) and abnormal cerebrospinal fluid (n = 16). A further 14 patients with cerebrospinal fluid shunts but no abnormality in the cerebrospinal fluid provided a control group for α-interferon determinations. The group with viral meningitis and the group with abnormal cerebrospinal fluid had significantly higher α-interferon concentrations ($p < 0.001$) when compared with those of the three other groups. This assay had great predictive value in determining those patients with abnormal cerebrospinal fluid who did not have a bacterial cause of meningitis. As the groups with abnormal cerebrospinal fluid and viral meningitis had a similar spread in α-interferon values it is likely that both reflect viral infection of the central nervous system.

Viruses are a major cause of meningitis,[1] yet the usefulness of the virological examination of cerebrospinal fluid has been questioned.[2] This is because the aetiological agent of an aseptic meningitis may only be identifiable in 20% of cases. Recently interferon in serum and cerebrospinal fluid has been shown to have high specificity for viral infections[3 4] and was present despite the absence of specific neutralising antibody.[5] In only one of these studies,[4] however, was cerebrospinal fluid from cases of bacterial meningitis (two cases) tested for viral pathogens, and thus the previous reports of high concentrations of interferons in patients with bacterial meningitis[6–9] cannot be fully substantiated.

Patients with meningitis pose important diagnostic and management problems. An early diagnosis of viral meningitis can result in a reduced hospital stay and prevent the prolonged use of antibiotics.[2] The objective of this study was to evaluate the diagnostic value of α-interferon concentrations, using a highly specific monoclonal antibody technique[10] in 100 patients in whom meningitis was clinically suspected.

Patients and methods

This study was conducted prospectively over the winter months on patients with clinically diagnosed meningitis and in whom enough cerebrospinal fluid remained after routine isolation procedures for bacterial and viral pathogens. Cerebrospinal fluid was examined for α-interferon in 100 patients (48 females and 52 males aged 2 months to 37 years) and a control group of 14 patients (six females: eight males aged 2 months to 15 years) with cerebrospinal fluid shunts but no cerebrospinal fluid abnormality. Virus isolation from cerebrospinal fluid samples was undertaken in human embryonic lung fibroblasts, HEp 2, and secondary monkey kidney cells. Virus infection of monolayers was detected using standard techniques, with specific neutralising sera to identify isolates. Suckling mice were not used in these investigations. Laboratory results were then compared with clinical information and routine biochemical, bacteriological, and viral analysis of the cerebrospinal fluid. Other relevant information to the diagnosis of meningitis, such as positive serology or viral isolation from a sample of faeces, was also considered. The α-interferon concentrations in the cerebrospinal fluid of a further 14 patients with cerebrospinal fluid shunts were determined. As this group of patients were asymptomatic, had normal cerebrospinal fluid findings, and negative bacteriological and viral culture they were regarded as a control group.

The α-interferon assay was a two site immunoradiometric (IRMA) assay (Boots-Celltech), which was carried out according to the manufacturer's

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Table 1 *Results of cerebrospinal fluid examination in normal cerebrospinal fluid** or predominant cell type in abnormal cerebrospinal fluid****

	Identified				*Not identified*				*No pathogen*	
	Bacterial		*Viral*		*Abnormal cerebrospinal fluid*		*Normal cerebrospinal fluid*		*Normal cerebrospinal fluid*	
No of cases	12		15		16		57		14	
IFN-α result*	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Normal cerebrospinal fluid	0	0	1	0	0	0	1	56	0	14
No with predominant neutrophils	0	11	2	0	3	1	0	0	0	0
No with predominant lymphocytes	1	0	11	1	10	2	0	0	0	0
Ratio of positive IFN-α results	1/12 (8·3%)		14/15 (93·3%)		13/16 (81·3%)		1/57 (1·8%)		0/14 (0%)	

*α-interferon (+) when result > 1·5 IU/ml; **(≤9 × 10^6/l); ***≥10 × 10^6/l).

instructions. In this assay α-interferon in the clinical specimen is captured by a radiolabelled highly specific monoclonal antibody (designated YOK 5/19 to IFN-α)[10] and immobilised using a solid phase on which sheep anti-α-interferon is covalently coupled and provides a second epitope for α-interferon. The interassay and intra-assay reproducibility was confirmed in a series of 20 samples (0–100 IU/ml) to have a coefficient of variation of < 10%. In a series of additional experiments where specific neutralisation of α-interferon was carried out, a cut off of 1·5 IU/ml was determined by sensitivity and specificity evaluations.

Results

Based on the results of the cerebrospinal fluid cell count and the bacteriological and viral culture of the cerebrospinal fluid, five patient groups were identified: normal CSF (normal CSF count, negative bacteriology and virology, 26 females: 31 males, mean (SD) age 9·7 (10) years; bacterial meningitis (cerebrospinal fluid count ≥10 × 10^6/l, bacteria isolated from cerebrospinal fluid or other evidence of bacterial cause, such as blood culture, eight females: four males mean age 9·3 (13·5) years; viral meningitis (virus grown from cerebrospinal fluid or cerebrospinal fluid count ≥10 × 10^6/l and other evidence of viral infection, such as positive serology, eight females: seven males mean age 7·4 (7·9) years; abnormal cerebrospinal fluid of unknown cause (cerebrospinal fluid count ≥10 × 10^6/l, bacteriological and viral culture negative, six females: 10 males, mean age 8·0 (8·4) years; and normal cerebrospinal fluid (patients with cerebrospinal fluid shunts and normal cerebrospinal fluid counts and no growth on bacteriological or viral culture, six females: eight males, mean age 5·1 (5·5) years. The bacterial causes of meningitis were: *Neisseria meningitidis* (n = 4), *Haemophilus influenzae* (n = 2), *Streptococcus pneumoniae* (n = 1), *Pseudomonas* sp (n = 1), *Klebsiella* sp (n = 1), coliforms (n = 1), *Mycobacterium tuberculosis* (n = 1) and *Listeria monocytogenes* (n = 1). Viral causes of meningitis were: echovirus type 7 (n = 8), type 9 (n = 2), type 11 (n = 1), type 14 (n = 1), coxsackie A9 (n = 1), coxsackie B5 (n = 1) and mumps (n = 1). In the group with bacterial meningitis 11 patients had a cerebrospinal fluid with a predominant neutrophil count and in one case (*Listeria monocytogenes*) lym-

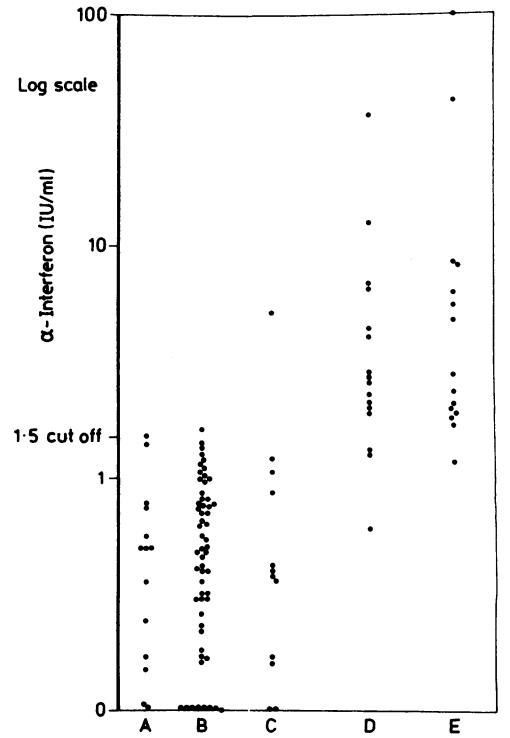

Fig 1 *Alpha-interferon concentrations in cerebrospinal fluid of five groups of patients (A) = 14 control patients; (B) = 57 patients with normal cerebrospinal fluid and meningitis; (C) = 12 patients with bacterial meningitis; (D) = 16 patients with abnormal cerebrospinal fluid and meningitis; (E) = 15 patients with viral meningitis.*

Table 2 *Studies of patients with confirmed bacterial meningitis and those with raised interferon concentrations in cerebrospinal fluid*

Study	*Patients examined*	*Raised interferon (%)*
Gresser and Naficy[9]	25	3 (12)
Larke[8]	34	1 (3)
Flowers and Scott[4]	2	0 (0)
Present study	12	1 (8)

phocytes predominated; in the group with viral meningitis 12 had a predominance of lymphocytes, two had predominant neutrophils, and one had a normal cerebrospinal fluid count. In the patients with abnormal cerebrospinal fluid (unknown cause) 12 had high lymphocyte counts and four high neutrophil counts. Table 1 shows the relation between α-interferon in each group and the cerebrospinal fluid cell predominance.

Fig 1 shows the results of the α-interferon assays. Using the Wilcoxon rank sum test, the viral meningitis and abnormal cerebrospinal fluid groups were significantly different from the three other groups ($p < 0{\cdot}001$). There was no significant difference between the groups with viral meningitis or abnormal cerebrospinal fluid, nor between the groups with normal cerebrospinal fluid, bacterial meningitis, and the controls. There was no association between the interferon concentrations and date of onset, age of patient, sex, white cell (or lymphocyte) count, glucose, protein or lactate concentrations in the cerebrospinal fluid.

Discussion

The methods used to measure interferon have been considerably improved. Until recently studies have used the plaque inhibition assay[4 6-9] or a dye binding semi microassay.[3] The IRMA assay, using a monoclonal antibody, which was also used in this study, is not only more sensitive but is also much more rapid. Previous methods took two days and normal ranges were considered to be $\leqslant 10$ IU/ml[4] or $\leqslant 12$ IU/ml[3]. The method used in this paper took six hours, and normal ranges for control samples of cerebrospinal fluid were $\leqslant 1{\cdot}5$ IU/ml. Of our 28 abnormal interferon results, 24 were in the range 1·5 to 10 IU/ml. The increased sensitivity of the test, therefore, permitted the detection of abnormal interferon concentrations, which would have been previously classified as normal because of limitations in methodology. A rapid analysis of the cerebrospinal fluid also gives considerable advantages to the clinician in his decisions on the immediate management of the patient.

A major clinical problem is the differentiation of bacterial and viral meningitis. One study[7] showed raised cerebrospinal fluid interferon concentrations in 28 of 53 (52%) patients with bacterial meningitis. That study was atypical in several ways: eight of the 28 (29%) were fatal cases (four samples were obtained at necropsy), and 16 of 28 (57%) had a meningitis secondary to another disease (six malignancies), surgical, or diagnostic procedure. Viral culture was not performed, and it is impossible to determine how many patients had a coincident viral infection. Furthermore, the results of other studies have not supported these findings (table 2). If our study and those of other workers[4 8 9] are all taken together, there is an overall raised interferon concentration in only 8% of patients (table 2). Of these five patients with bacterial meningitis, the organism responsible was *Haemophilus influenzae* in three and *Listeria monocytogenes* in two cases. In our study the two patients with *H influenzae* meningitis did not have raised α-interferon concentrations, but the one case of α-interferon (positive) bacterial meningitis was associated with *L monocytogenes* infection, as seen in one of the other studies.[9] Interestingly, both of these patients had a cerebrospinal fluid in which lymphocytes were predominant. Thus it seems that raised interferon concentration in patients with bacterial meningitis is uncommon, but if found, the causative agent is likely to be *H influenzae* or *L monocytogenes*.

The objective of this study was to test the diagnostic value of the α-interferon assay applied to cerebrospinal fluid specimens. At present, the differential cell count, glucose, protein, and lactate concentrations are considered to be useful in the predictive differentiation of bacterial as opposed to viral meningitis.[11] In our cases the results of α-interferon in cerebrospinal fluid were a better guide to viral meningitis than all other variables, including differential cell count in cerebrospinal fluid (table 1). This is probably because early in viral meningitis there may be a predominance of neutrophils. In the patients with abnormal cerebrospinal fluid findings 13 had raised α-interferon concentrations, and it is likely that these patients had a viral meningitis. If this is so α-interferon determinations may be a better indicator of viral infection of the central nervous system than virus isolation alone. We do not agree with Flowers and Scott[4] that, "it is of no interest to the clinician if interferon is found to be present but it is subsequently not possible to identify a putative virus." We feel that clinicians will find it useful to have another measurement of cerebrospinal fluid which is more sensitive than virus isolation from the cerebrospinal fluid and more predictive of central nervous system infection than that of isolation from other sites.[12]

In our opinion the determination of α-interferon provides a useful discrimination between viral and

bacterial meningitis and is a useful adjunct to the virological tests currently used in diagnosing infection of the central nervous system.

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