Cerebrospinal fluid analysis differentiates between relapsing-remitting and secondary progressive multiple sclerosis

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Abstract

Objectives—To find whether CSF analysis may differentiate between relapsing-remitting and secondary progressive multiple sclerosis.

Methods—In 17 patients with relapsing-remitting and 16 patients with secondary progressive multiple sclerosis, all without current or recent relapses, albumin CSF: peripheral blood ratio, mononuclear cell number, CD4+, CD8+, and B1+ subsets, CD4+:CD8+ ratio, IgG, IgG index, IgM, IgM index, complement components C3 and C4, and C3 and C4 indices, myelin basic protein, neuron specific enolase, S100, and lactate were determined. For each measure the statistical distance measure D^2 was calculated. For computation of a discriminant score variables with a P value ≤ 0.15 were included (two sided univariate t test). These were albumin CSF: peripheral blood ratio, mononuclear cell number, IgM, IgM index, C3, C4, neuron specific enolase, S100, and lactate. Simultaneous distributions of the variables were compared between both groups (multivariate t test) and a discriminant score was computed (linear discriminant analysis).

Results—The discriminant score allocated all 14 relapsing-remitting patients to the relapsing-remitting group (positive score) and 12 of 13 secondary progressive patients to the secondary progressive group (negative score). One secondary progressive patient was allocated to the relapsing-remitting group.

Conclusions—Patients with relapsing-remitting or secondary progressive multiple sclerosis differ in CSF profile and CSF analysis may help to differentiate between relapsing-remitting and secondary progressive multiple sclerosis.

(*J Neurol Neurosurg Psychiatry* 1997;63:446-451)

Keywords: multiple sclerosis; cerebrospinal fluid; discriminant analysis

Multiple sclerosis is a presumed autoimmune disease of the CNS with focal inflammation and demyelination as the major pathological features. The pathogenesis of CNS lesions consists of a series of events. Increased permeability of the blood-brain barrier enables

mononuclear cells to enter the CNS parenchyma and to form perivascular infiltrates.^{1,2} The immune mechanisms then occurring involve T cells, macrophages, and antibodies.¹⁻³ The inflammatory immune attack is thought to result in demyelination.⁴ Whereas inflammation is not necessarily associated with structural damage, demyelination leads to persistent deficits, producing a permanent interruption in axonal conduction.^{1,4} Later in the disease process axonal and glial damage also occur.^{1,5}

Basically, these processes are reflected by changes in the composition of the CSF. Thus an increased CSF:peripheral blood ratio for albumin represents increased permeability of the blood-brain barrier (transudation), and raised mononuclear cell numbers in CSF reflect the cellular component of the inflammation.⁶ The presence of CSF specific oligoclonal bands and increased IgG and IgM indices indicate intrathecal immunoglobulin production.⁶ In addition, intrathecal activation of the complement system leads to altered CSF concentrations of the complement components C3 or C4 or their intrathecal synthesis.^{7,8} Increased CSF concentration of myelin basic protein mirrors demyelination.⁹ Myelin basic protein values are raised in patients with clinically active multiple sclerosis and fall significantly after methylprednisolone treatment.¹⁰ Neuron specific enolase is a marker for neuronal damage and CSF concentrations are increased in various disorders of the CNS.¹¹⁻¹³ The nervous system specific S100 protein is notably present in astrocytes and oligodendrocytes, and CSF S100 is a marker for glial damage.^{11,12,14-16} In patients with multiple sclerosis S100 concentrations are higher during active disease.^{14,15} Subsequently, the CSF lactate concentration increases as a result of intracerebral processes involving ischaemia.^{6,17}

Most patients with multiple sclerosis start with a relapsing-remitting course. After five to 10 years most patients gradually transgress to the secondary progressive phase.⁵ Histopathological and MRI evidence indicate that pathogenic and pathological differences exist between relapsing-remitting and secondary progressive multiple sclerosis.^{18,19} By contrast with the relapsing-remitting phase the progressive phase shows less evidence for an autoimmune process.⁵ Data from MRI and NMR spectroscopy show that the chronic progression is related to neuronal and axonal

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Received 10 February 1997
and in revised form 21 April
1997

Accepted 6 May 1997

Table 1 Cerebrospinal fluid measures in relapsing-remitting (RR) and secondary progressive (SP) patients

Units	Median values		Reference values	RR v SP	
	RR (n=17)	SP (n=16)		D ²	P value
Transudation:					
Albumin CSF:PB	4.3	5.0	1.8–7.0	0.39	0.09
Cellular immunology:					
Mononuclear cells	μl	3	0–4	0.61	0.03
CD4+	%	71	50–80	0.03	0.63
CD8+	%	20	15–37	0.004	0.86
CD4+/CD8+		3.4	1–4	0.001	0.96
B1+	%	3	0–5	0.06	0.50
Humoral immunology:					
IgG	mg/l	37	11–38	0.11	0.36
IgG index		0.83	0.36–0.60	0.09	0.79
IgM	mg/l	0.6	0.1–0.4	0.36	0.10
IgM index		0.07	0.01–0.07	0.27	0.15
Complement C3	mg/l	2.0	0.9–6.7	0.41	0.08
Complement C3 index		0.57	0.32–0.77	0.004	0.86
Complement C4	mg/l	1.3	0.6–3.5	0.28	0.14
Complement C4 index		0.92	0.4–1.7	0.04	0.58
Demyelination:					
MBP	μg/l	0.6	0.2–1.2	0.19	0.22
Neuronal damage:					
NSE	μg/l	6.2	2.0–10.0	0.30	0.12
Glial damage:					
S100	μg/l	1.5	0.7–2.0	0.29	0.13
Metabolic change:					
Lactate	μmol/l	1660	1380–1900	0.45	0.08

Median values, reference values, and statistical distance D² are given for each variable. PB = peripheral blood; MBP = myelin basic protein; NSE = neuron specific enolase.

damage.^{5 18 19} In view of these differences, drugs that are effective in the relapsing-remitting phase are not necessarily so in the secondary progressive phase, and drug research involves separate trials for relapsing-remitting and secondary progressive multiple sclerosis. Thus the immunomodulating agent copolymer-1 has been shown to lessen disability in relapsing-remitting patients but was not effective in the chronic progressive phase.^{20 21} As the number of immunotherapies is expected to increase in the near future, accurate distinction between relapsing-remitting and secondary progressive multiple sclerosis becomes more relevant, to optimise the application of possibly expensive drugs. Importantly, however, at a certain stage of the disease course various circumstances may seriously hinder the differentiation between relapsing-remitting and secondary progressive multiple sclerosis—namely, clinical fluctuations in between relapses in the relapsing-remitting phase; the insidious character of the transgression to the secondary progressive phase; and the occurrence of stable periods in the secondary progressive phase. As a consequence, in individual patients the secondary progressive phase of the disease can often only be diagnosed reliably in retrospect.

Considering the above, we studied whether detailed CSF examination might differentiate between relapsing-remitting and secondary progressive multiple sclerosis. We measured a range of CSF variables in patients with clinically established relapsing-remitting or secondary progressive multiple sclerosis, and used discriminant analysis to develop an allocation index that distinguishes between the relapsing-remitting and secondary progressive type.

Patients and methods

PATIENTS

Thirty three patients were studied, 17 having been diagnosed as having relapsing-remitting disease and 16 as having secondary progressive disease on clinical grounds, in accordance with a recent consensus report.²² Inclusion criteria were (a) clinically definite multiple sclerosis,²³ (b) for relapsing-remitting patients: two or more relapses in the preceding two years, and (c) for secondary progressive patients: at least six months of continuous progression in the year before the study, with or without relapses. Exclusion criteria were (a) relapse in the three months before the study, (b) corticosteroid treatment in the two months before the study, (c) any previous immunosuppressive treatment, including cyclophosphamide, azathioprine, and cyclosporine A, and (d) contraindication for lumbar puncture. In each patient age, disease duration, number of relapses, and total methylprednisolone dose in the two years before the study were ascertained. In secondary progressive patients the duration of the secondary progressive phase was determined. On neurological examination one of us (ORH), an experienced neurologist, determined the expanded disability status scale (EDSS) score (24) with a 0.5 point interval—based on assessment of the neurological (Kurtzke) and the Scripps neurological rating scales (SNRS).^{24 25} The progression rate was calculated as the EDSS score:disease duration ratio. The study protocol was approved by the ethics committee of the University Hospital Nijmegen. Written informed consent was obtained from each patient.

CEREBROSPINAL FLUID EXAMINATIONS

Seven types of pathogenic or pathological processes were considered as potentially different between the relapsing-remitting and the secondary progressive phase of the disease: blood-brain barrier disturbance, cellular immune processes, humoral immune processes, demyelination, neuronal damage, glial damage, and metabolic changes. Measurements in CSF corresponding to these processes were albumin CSF:peripheral blood ratio, mononuclear cell number, CD4+, CD8+, and B1+ subset percentages, CD4+:CD8+ ratio, IgG, IgG index, IgM, IgM index, complement component C3, complement component C3 index, complement component C4, complement component C4 index, myelin basic protein, neuron specific enolase, S100, and lactate. Lumbar punctures were performed at level L5-S1 according to standard procedures with an atraumatic conical elliptic shaped spinal needle 22G 103 mm with introducer (Poli-med). All punctures were performed between 1100 and 1300 hours and about 10 ml colourless CSF was obtained. Simultaneously, peripheral venous blood was drawn via cubital venapuncture. Cell counting and determination of lymphocyte subpopulations were immediately performed. For the remaining measurements paired samples of CSF and serum were stored at –70°C. The lymphocyte subsets were assayed by flow cytometry on a FACS II cell

Table 2 Characteristics of study population

	Relapsing-remitting (n=17)	Secondary progressive (n=16)	P value t test
M/F	5/12	8/8	
Age (y)	36.2 (8.1)	40.2 (7.5)	0.15
EDSS	3.0 (1.1)	4.1 (1.3)	0.01
Scripps NRS	72.7 (12.5)	62.1 (12.2)	0.02
Relapses in 2 years before study	2.8 (0.8)	0.6 (0.8)	0.0001
Disease duration (y)	6.4 (4.3)	9.2 (3.8)	0.06
Progression rate	0.7 (0.6)	0.5 (0.2)	0.18
Progressive phase (y)		3.9 (1.9)	

EDSS = expanded disability status scale; NRS = neurological rating scale.

Values are given as mean (SD).

sorter (Becton Dickinson, USA) according to a method described by Dux *et al.*²⁶ CD3+, CD4+ (helper-inducer cell), CD8+ (suppressor-cytotoxic cell) and CD20+ (B1 cell) were analysed. Fluorescein labelled antibodies were purchased from Dakopatt (Denmark). For distinguishing lymphocytes from other cells (erythrocytes) the lymphocyte marker CD45 CYQ (IQP, Groningen, The Netherlands) was always included in each test. IgG, IgM, and albumin were determined by immunonephelometry on a COBAS FARA II apparatus (Hoffman La Roche, Switzerland) with the exception of CSF IgM, which was assayed by an enzyme linked immunosorbent assay (ELISA) method as described by Jongen *et al.*^{27, 28} Complement components C3 and C4 were measured by immunonephelometry on a Hyland laser nephelometer using antiserum from Dakopatt (Denmark).²⁷ Myelin basic protein was determined by a double antibody radioimmunoassay kit according to the instructions of the manufacturer (Diagnostic Systems Laboratories, Webster Texas, USA).²⁹ Neuron specific enolase measurement was performed by an immunoluminometric assay on an LIA-MAT analyser according to the instructions of the manufacturer (Sangtec Medical, Sweden). Age related values for these CNS specific proteins in CSF in our laboratory have been published.³⁰ S100 was determined by a double monoclonal antibody enzyme immunoassay according to the procedure published by Lamers *et al.*¹⁷ The index value of a distinct protein = protein CSF×albumin serum:protein serum×albumin CSF. Table 1 presents the reference values for CSF indices (P5-P95), as established in our laboratory.

STATISTICAL ANALYSIS

For the 18 CSF variables univariate and multivariate statistical analyses were performed after logarithmic transformation.³¹ For each CSF variables (table 1) the median value was calculated for both groups (relapsing-remitting and secondary progressive) by interpolation from the cumulative sample distributions. To rank the indices after their discriminating power (between relapsing-remitting and secondary progressive) the usual statistical distance measure D^2 was calculated,

$$D^2 = ((\text{mean}(\log(x)_{RR}) - \text{mean}(\log(x)_{SP})) / SD_p)^2$$

with SD_p being the pooled SD (after logarithmic transformation) over both groups.

Figure 1 Cerebrospinal fluid discriminant score values in 17 patients with relapsing-remitting (RR) and 16 patients with secondary progressive (SP) multiple sclerosis.

For each variable the statistical comparison of the sample distribution between both groups was done by univariate two sample *t* test (two sided $\alpha=0.05$). For the computation of an allocation index (=discriminant score) we tried to use fewer variables. This was achieved by inclusion only of those variables for which the *P* value of the two sided univariate *t* test was ≤ 0.15 . The simultaneous distributions of these variables were compared between both groups by means of the multivariate *t* test (Hotelling's test). A discriminant score was computed according to the method of linear discriminant analysis (Fisher's equal probabilities). A second discriminant analysis was performed on those selected variables assumed to be determinable at routine laboratories.

Results

PATIENT CHARACTERISTICS

Table 2 shows patient characteristics. As a group secondary progressive patients had significantly higher EDSS scores, lower SNRS scores, and a lower number of relapses than relapsing-remitting patients. All but three patients—one relapsing-remitting and two secondary progressive—had received methylprednisolone in the two years before the study. No patient had received prednisone in these years. The total methylprednisolone doses in the two years before the study did not differ significantly between relapsing-remitting and secondary progressive patients.

DISCRIMINANT ANALYSIS

Table 1 shows median values of CSF variables, reference values, D^2 after logarithmic transfor-

Table 3 Calculation of CSF discriminant scores in two patients

Variable	Value	Log	Multiplication factor	Contribution to discriminant score	Cumulative score
Patient A					
Intercept				-5.95	-5.95
Q albumin	4.30	1.4586	14.37	20.96	15.01
MNC	11.00	2.3979	1.15	2.75	17.76
IgM	0.30	-1.2040	6.13	-7.38	10.37
IgM index	0.07	-2.6593	-4.25	11.30	21.76
C3	1.70	0.5306	-21.02	-11.15	10.52
C4	0.60	-0.5108	-4.52	2.31	12.83
NSE	7.10	1.9604	6.02	11.80	24.63
S100	0.66	-0.4186	2.23	-0.93	23.70
Lactate	1832	7.5132	-2.66	-20.02	3.68
Discriminant score					+3.68
Allocation to RR					
Patient B					
Intercept				-5.95	-5.95
Q albumin	16.90	2.8273	14.37	40.63	34.68
MNC	9.00	2.1972	1.15	2.52	37.19
IgM	2.40	0.8755	6.13	5.37	42.56
IgM index	0.10	-2.3026	-4.25	9.78	52.34
C3	7.00	1.9459	-21.02	-40.90	11.45
C4	3.20	1.1632	-4.52	-5.26	6.19
NSE	5.23	1.6536	6.02	9.95	16.14
S100	0.71	-0.3496	2.23	-0.78	15.36
Lactate	1971	7.5863	-2.66	-20.21	-4.85
Discriminant score					-4.85
Allocation to SP					

MNC = Mononuclear cells; C3 = complement component C3; C4 = complement component C4; NSE = neuron specific enolase; Q albumin = CSF: peripheral blood albumin ratio.

mation, and *t* test *P* values. Based on *P* values the following variables were selected for discriminant analysis: CSF:peripheral blood albumin ratio (*P*=0.09), mononuclear cell number (*P*=0.03), IgM (*P*=0.10), IgM index (*P*=0.15), complement component C3 (*P*=0.08), complement component C4 (*P*=0.14), neuron specific enolase (*P*=0.12), S100 (*P*=0.13), and lactate (*P*=0.08). Discriminant analysis resulted in a discriminant score (allocation index) composed of these nine variables. Using this score all 14 relapsing-remitting patients were allocated to the relapsing-remitting group (positive discriminant score), and 12 of 13 secondary progressive patients to the secondary progressive group (negative discriminant score). One secondary progressive patient was allocated to the relapsing-remitting group. Table 3 shows examples of calculation of discriminant scores. Figure 1 shows the discriminant scores for the 33 patients. Notably, inclusion of nine variables by means of a stepwise discriminant analysis (significance levels "entry" and "stay" both 0.15)—in which only patients were included with complete information on all variables—resulted in almost the same set of variables. Only lactate was replaced by myelin basic protein, probably due to leaving out five patients with missing values on lactate. A second discriminant analysis was performed on those selected variables assumed to be determinable at routine laboratories. This analysis included only the CSF variables CSF:peripheral blood albumin ratio, mononuclear cell number, IgM, IgM index, and lactate. The scores of the secondary progressive group overlapped those of the relapsing-remitting group, as seven of the 13 secondary progressive patients had a score falling in the relapsing-remitting range (fig 2).

Figure 2 Values of a restricted CSF score in 17 patients with relapsing-remitting (RR) and 16 patients with secondary progressive (SP) multiple sclerosis. Values were computed from variables considered to be determinable at routine laboratories (albumin ratio, mononuclear cell number, CSF IgM, IgM index, and CSF lactate).

Discussion

Recent reports underline the importance of CSF analysis in the differential diagnosis of CNS disorders.^{32,33} The combination of several CSF measurements greatly improves the differential diagnosis of multiple sclerosis versus other neurological disorders and CSF analysis supports the diagnostic classification of multiple sclerosis.^{23,34} We investigated whether detailed CSF analysis may differentiate between the relapsing-remitting and secondary progressive phase of the disease, and we performed a discriminant analysis of CSF variables in these patients. The variables studied reflected transudation, cellular and humoral immune processes, demyelination, neuronal and glial damage, and hypoxia. The discriminant score allocated 26 out of 27 patients to either the relapsing-remitting or secondary progressive group in agreement with the clinical diagnosis. This finding implies that patients diagnosed as having a relapsing-remitting or secondary progressive course on clinical grounds also differ with respect to CSF profile, and that the discriminant score may be of help in classifying individual patients as being relapsing-remitting or secondary progressive.

An important aspect of our study is that we only included relapsing-remitting patients who had had no current or recent clinical relapses. So our data are not confounded by abnormalities resulting from events related to exacerbation. On follow up the first exacerbations occurred at a mean of 137 (SD 103) days after

CSF sampling, the earliest relapse occurring at 41 days, for which reason it is unlikely that CSF findings reflect a subclinical increase of disease activity.

Pathological and MRI studies in multiple sclerosis suggest that various pathogenic and pathological processes may be at work simultaneously.^{5 18 19} Conceivably, the activity of each of these processes in the secondary progressive phase may be only slightly different from that in the relapsing-remitting phase, which means that for a given variable CSF concentrations in the secondary progressive phase may not differ significantly from those in the relapsing-remitting phase. For this reason we did not search for a single variable that might differentiate between the two groups; nor did we try to detect significances for single variables. These might be found in large study populations, but, as expected, individual values largely overlap and are of little use as a discriminating tool. By contrast, our study was designed to detect changes in a complex CSF profile in a relatively small group of patients and thus to develop a discrimination index that may be used in individual patients. Thus we found that nine of the 18 variables tested (50%) had a P value ≤ 0.15 and thus contributed to the discrimination index. As CSF measurement of neuron specific enolase, S100, and the complement components C3 and C4 may not be performed routinely in hospital laboratories, we determined the discriminating value of the CSF:peripheral blood albumin ratio, mononuclear cell number, IgM, IgM index, and lactate. This second score seemed to be of limited use given that seven of the 13 secondary progressive patients had scores in the relapsing-remitting range.

The establishment of a relation between a clinical variable of multiple sclerosis and changes of CSF mononuclear cells, immunoglobulins or complement components is beset with difficulties, as immunotherapies may have long lasting effects on CSF immune indices.³⁵ In our study no patient had received any cytotoxic agent, nor had recently used corticosteroids. Moreover, the total steroid dose in the two years before the study did not differ between relapsing-remitting and secondary progressive patients. For these reasons we think that we measured intrinsic differences and not treatment induced artifacts.

Generally, the relapsing-remitting patients had increased mononuclear cell numbers and IgM, as well as neuron specific enolase and S100. The results for IgM in these patients seem not to reflect simple blood-brain barrier damage, as the IgM index tended to be higher than in secondary progressive patients. Secondary progressive patients generally had increased lactate and albumin with consequent increases of C3 and C4, the last probably due to blood-brain barrier damage rather than local synthesis, as the indices are normal. The significantly lower mononuclear cell numbers in the CSF of secondary progressive patients may indicate that the inflammatory component of the disease "burns out" during its natural course. Our findings on the albumin ratio tend

to suggest that damage to the blood-brain barrier in the secondary progressive phase is rather more diffuse than the more striking punctate abnormalities which are seen in gadolinium MRI studies of the relapsing-remitting form. Others have also shown that the impairment of the blood-brain barrier in secondary progressive patients is worse than in relapsing-remitting patients.³⁶ For the finding that CSF IgM in secondary progressive patients tended to be lower than in relapsing-remitting patients (means 0.4 v 0.6 , $P=0.10$), Sindic *et al* reported a raised IgM index in 32% of 80 patients with multiple sclerosis, but in none of 10 patients with a history of multiple sclerosis exceeding 15 years.³⁷ Two other studies registered raised IgM indices at a decreasing rate with an increasing duration of disease.^{38 39} Although the lower IgM values in our secondary progressive patients may partly relate to duration of disease, the discriminant score was not related to disease duration (data not shown).

Concentrations of C4 in CSF made a large contribution to the differentiation index in that lower C4 is related to the relapsing-remitting course. As early as 1965 it was shown that CSF complement activity is lower in patients with multiple sclerosis than in controls, and the lowest during acute deterioration.⁴⁰ Moreover, patients with raised CSF γ -globulin content showed lower complement activity than those with normal CSF γ -globulin, and the complement activity was significantly correlated with the total protein concentration in controls, but not in the patients with multiple sclerosis.⁴⁰ Recent CSF studies in patients with acute optic neuritis and multiple sclerosis confirmed previous reports on intrathecal complement activation.⁸ Therefore, we think that one explanation for the relatively low C4 concentrations in our relapsing-remitting group may be steady C4 consumption by immune mediated processes in between relapses. On the other hand, it is conceivable that C4 discrepancies reflect immunogenetic differences. In a recent study on complement gene polymorphisms Franciotta *et al* reported that patients with relapsing-remitting multiple sclerosis have a significantly higher frequency of C4AQ0 (null) allele than primary progressive patients.⁴¹ Moreover, a structural gene deletion was present in 45% of relapsing-remitting patients with the C4AQ0 allele.⁴¹ As complement factors contribute to elimination of immune complexes, deficiency of a functional complement component could predispose to multiple sclerosis, and more specifically to the occurrence of virus induced relapses.

The CNS tissue markers S100 and neuron specific enolase also contributed to the discriminant score, in that high CSF concentrations suggested relapsing-remitting diagnosis. As our patients were sampled in a relapse free period, this finding suggests that in between relapses tissue damage is more pronounced than in the progressive phase. Recently Massaro *et al* performed a five week follow up study of CSF changes during and after an exacerbation; S100 concentrations increased early after

relapses and remained increased during the whole study period.¹⁵

To conclude, we calculated a discriminant score that differentiates between relapsing-remitting and secondary progressive multiple sclerosis. From a pathogenic and pathophysiological point of view, our data indicate that a combination of cellular and antibody mediated, immune processes involving complement lead to damage of neurons and glia, and that these processes are more pronounced in relapsing-remitting than in secondary progressive multiple sclerosis. We speculate that monitoring of the discriminant score will prove useful in the early and accurate diagnosis of the secondary progressive versus the relapsing-remitting phase in individual patients. However, as we included in our study only patients with an unquestionable clinical course, prospective studies are needed to validate the discriminant score and to assess its applicability in patients just transgressing from the relapsing-remitting to the secondary progressive phase.

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