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## 14-3-3 protein in the CSF as prognostic marker in early multiple sclerosis

**Article abstract**—Axonal damage probably occurs early in the evolution of MS. Five of 38 (13%) patients had a positive assay for the neuronal 14-3-3 protein in the CSF obtained at the first clinically isolated syndrome suggestive of MS. A positive 14-3-3 assay was the only independent predictor for a shorter time to conversion to clinical definite MS (risk ratio 4.1; 95% CI 1.1 to 15) and to reach an Expanded Disability Status Scale (EDSS)  $\geq 2$  at the end of follow-up (odds ratio 14.8; 95% CI 2.86 to 76.8). The detection of the 14-3-3 protein in the CSF at the first neurologic event suggestive of MS may be a useful predictor of short-term evolution.

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In a given patient with a first clinically isolated syndrome suggestive of MS, the prediction of the clinical evolution remains a challenge. Brain MRI and CSF oligoclonal IgG bands are helpful to identify which patients are at risk of developing clinically definite MS (CDMS).<sup>1</sup> However, how soon patients will have a second relapse or which patients will have early functional impairment cannot be predicted with accuracy.

Axonal damage is recognized as part of the MS lesion and probably occurs early in the evolution of the disease.<sup>2</sup> We hypothesized that patients at risk of early disability or with higher relapse rate could be those with more prominent or persistent axonal damage at the onset of MS. At present, there are no biologic markers of axonal damage in MS. In this study, we analyzed whether the detection of 14-3-3 protein and S100 $\beta$  in the CSF obtained at the first clinically isolated syndrome suggestive of MS might serve as markers of early axonal injury and predictors of the clinical outcome of these patients. Both proteins are increased in the CSF of neurologic disorders associated with subacute, usually severe, neuronal damage.<sup>3–6</sup>

ers associated with subacute, usually severe, neuronal damage.<sup>3–6</sup>

**Patients and methods.** We retrospectively analyzed the presence of 14-3-3 and S100 $\beta$  in the CSF of 38 patients obtained within 30 days of a first clinically isolated syndrome suggestive of MS as part of the diagnostic workup. Patients were followed in the MS units of the two participating hospitals and did not receive any specific treatment except steroids during relapses. A relapse was defined as the occurrence of new neurologic symptoms or the worsening of previous symptoms that lasted more than 24 hours and that stabilized or resolved either partially or completely. CSF samples were kept frozen at  $-80^{\circ}\text{C}$  until the time of analysis. The baseline characteristics of the patients are summarized in table 1.

The presence of 14–3–3 protein was analyzed by immunoblot using an anti-14-3-3 antibody reactive against all isoforms (no. Sc-629; Santa Cruz Biotechnology, Santa Cruz, CA) as previously described.<sup>3</sup> S100 $\beta$  was measured by an immunoluminometric method following the manufacturer's guidelines (LIA-mat Sangtec 100, Bromma, Sweden). CSF analysis was done blind to clinical, MRI, or CSF results in the same laboratory (Hospital Clinic).

**Statistical analysis.** Mann-Whitney exact-rank test for quantitative noncontinuous variables, Student's t-test for continuous variables and  $\chi^2$  test for categorical variables were used to study differences between the 14-3-3-positive and -negative groups. The time to the second relapse in both groups was compared using log-rank test. Cox proportional hazards regression model was used to verify associations between time to present a second neurologic event suggestive of MS (CDMS) and the following variables: age; sex; hospital; Kurtzke's Expanded Disabil-

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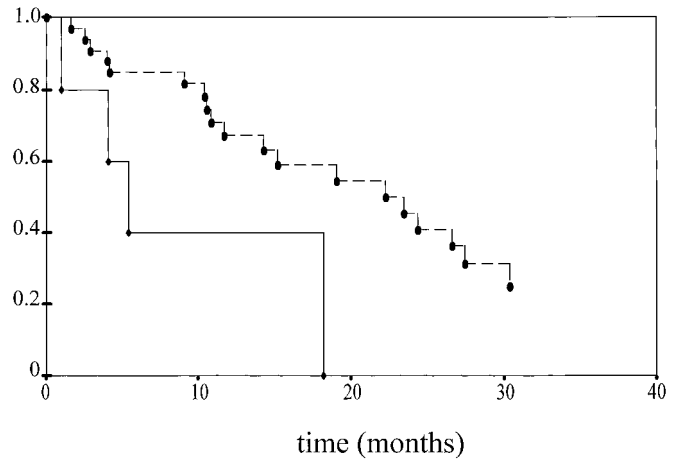
**Table 1** Baseline characteristics of the 38 patients with a first clinically isolated syndrome suggestive of MS

Characteristics	Value
Median age, y, (range)	32 (18–52)
Women, n (%)	25 (66)
Clinical syndrome	
Spinal cord	14 (38.2)
Optic neuritis	9 (23.5)
Brainstem	8 (20.6)
Supratentorial/other	7 (17.7)
EDSS, median (range)	3.0 (1.0–5.5)
MRI suggestive of MS, n (%)*	30 (79)
CSF OB or IgG index >0.7, n (%)	28 (73.7)
Steroid treatment at first relapse, n (%)	32 (84.6)
Median follow-up, mo, (range)	27.3 (6–68)

\* MRI suggestive of MS demonstrates four supratentorial lesions or three lesions, at least one infratentorial or gadolinium enhancing.

EDSS = Expanded Disability Status Scale, CSF OB = oligoclonal IgG bands.

ity Status Scale (EDSS) score at the time of the first event; MRI suggestive of MS (four supratentorial lesions or three lesions with at least one of infratentorial location or enhanced with gadolinium); presence of oligoclonal IgG bands, IgG index >0.7, albumin index >9.0, or pleocytosis in CSF; the use of corticosteroids in the first relapse; the results of the 14-3-3 assay; and the S100 $\beta$  and IgG levels in the CSF. A multivariate logistic regression was performed including the same variables to ascertain possible



*Figure.* Kaplan–Meier method shows the probability of no conversion to clinically definite MS in function of positive (solid line) or negative (broken line) 14-3-3 protein in CSF.

predictors for an EDSS  $\geq 2$  (evidence of at least mild disability) at the end of the follow-up.

**Results.** The 14-3-3 assay was positive in five patients (13.2%). There were no differences between 14-3-3-positive or -negative patients in age; sex; EDSS at onset; abnormal MRI; presence of oligoclonal IgG bands, elevated IgG index, elevated albumin index, or pleocytosis in CSF; and mean time of follow-up. The S100 $\beta$  levels were higher in the CSF of the 14-3-3-positive patients (table 2). The mean ( $\pm$  SD) decrease of the EDSS score shortly after the relapse, as a measure of recovery, was 0.8 (1.05) for the 14-3-3-positive group compared with 1.6 (1.8) for the 14-3-3-negative patients ( $p = 0.02$ ).

At the end of the follow-up, 80% of the 14-3-3-positive

**Table 2** Association between baseline and outcome characteristics and the presence of 14-3-3 protein in the CSF

Characteristics	14-3-3 in CSF		p Value
	Yes (n = 5)	No (n = 33)	
Median age, y (range)	34.1 (29–39)	31 (18–52)	NS
Women, n (%)	5 (100)	20 (60)	NS
EDSS at first event, median (range)	3.0 (2.0–4.0)	3.0 (1.0–5.5)	NS
MRI suggestive of MS, n (%)*	4 (80)	26 (79.7)	NS
CSF OB/IgG index >0.7, n (%)	5 (100)	23 (69.7)	NS
CSF pleocytosis, <15 cells/ $\mu$ L	0 (0)	3 (9)	NS
Albumin index >9.0 $\dagger$	0 (0)	2 (6)	NS
S100 $\beta$ , $\mu$ g/L, mean $\pm$ SD	1.4 $\pm$ 0.82	0.9 $\pm$ 0.38	0.04
Median time of follow-up, mo (range)	36 (7–39)	27 (6–68)	NS
Conversion to CDMS, n (%)	4 (80)	19 (57.5)	NS
Median time to second relapse, mo	5.4	23.4	0.01
No. of relapses, mean $\pm$ SD	3.6 $\pm$ 2.0	2.0 $\pm$ 1.3	0.03
EDSS $\geq 2$ at the end of follow-up, n (%)	4 (80)	7 (22)	0.02

\* MRI suggestive of MS demonstrates four supratentorial lesions or three lesions, at least one infratentorial or gadolinium enhancing.

$\dagger$  Albumin index >9.0 indicates disruption of the blood–brain barrier.

CSF OB = oligoclonal IgG bands; CDMS = clinically definite MS; EDSS = Expanded Disability Status Scale.

and 57.5% of the 14-3-3-negative patients developed CDMS. Compared with the 14-3-3-negative group, the 14-3-3-positive patients had a shorter time to conversion to CDMS (5.4 vs 23.4 months;  $p = 0.012$ ) (figure), a higher relapse rate (3.6 vs 2.0;  $p = 0.03$ ), and a higher frequency of patients with EDSS  $\geq 2$  at the end of the study (80% vs 22%;  $p = 0.02$ ) (see table 2).

In the multivariate analysis, the detection of the 14-3-3 protein in the CSF was the only variable associated with a shorter time to develop CDMS (risk ratio 4.1; 95% CI 1.1 to 15). Similarly, the presence of 14-3-3 protein in the CSF was an independent predictive factor for achieving an EDSS score  $\geq 2$  at the end of the follow-up (odds ratio 14.8; 95% CI 2.86 to 76.8).

**Discussion.** Our study shows that 13% of patients presented 14-3-3 protein in the CSF obtained at the first neurologic event suggestive of MS. The positive assay was associated with a shorter time to conversion to CDMS, a higher relapse rate, and an increased disability at the end of the follow-up.

The high proportion of patients who developed CDMS in our study (60%) agrees with the frequency of MRI that are suggestive of MS at the first event. Patients with an abnormal MRI at the time of presentation with a clinically isolated syndrome have a increased risk of developing CDMS over the next 5 years.<sup>1</sup> However, neither the MRI or presence of CSF oligoclonal IgG bands is a good predictive marker of early relapse or severity of neurologic dysfunction among those patients who develop MS.

Natural history studies of MS have identified early clinical and MRI variables that, when taken together, may predict the short-term outcome of a given patient and, with less accuracy, the long-term evolution. However, these predictors included, in addition to baseline characteristics such as age, clinical syndrome, and MRI status, variables such as frequency of relapses, interval between first two relapses, and recovery from initial attacks.<sup>7</sup> Our study also suggests that there is not a single clinical variable that predicts the clinical outcome, but we identified a CSF 14-3-3-positive assay as an independent prognostic factor for early relapse and disability in the short term.

The 14-3-3 family protein is expressed in the cell body and processes of neurons and may be detected in the CSF of patients with some neurologic disorders that cause subacute damage of the nervous system.<sup>3,4</sup> The detection of 14-3-3 protein in the CSF of patients with a first neurologic event suggestive of MS may reflect early neuronal injury. The correlation of the positive 14-3-3 assay with increased levels of S100 $\beta$  in the CSF<sup>6</sup> further supports this hypothesis. Although S100 $\beta$  is an astroglial marker, both proteins can be detected in conditions associated with neural damage. However, in our study, the only predictor of the clinical course was the 14-3-3 protein, as demonstrated by the multivariate analysis.

The 14-3-3 protein was analyzed in seven patients

with transverse myelitis of different origins and was detected in the CSF of the four patients who showed little or no recovery from their acute illness.<sup>5</sup> In our study, the presence of 14-3-3 protein in the CSF was not associated with the EDSS score during the first neurologic event but confirmed the correlation with a worse recovery. Moreover, the association between a positive CSF 14-3-3 assay and disability at the end of the follow-up would suggest that there is sustained axonal damage in absence of clinical activity. Because in the present study the lumbar puncture was only performed at the first neurologic event, we do not have evolutionary data supporting this hypothesis. However, this possibility is supported by recent data on brain atrophy in the follow-up of patients at risk of MS. A significant increase in ventricular size during the first year after the first neurologic event was only observed in those patients who developed CDMS compared with those who had no further symptoms.<sup>8</sup>

Recent studies demonstrated that early treatment with interferon beta delays the conversion to CDMS in patients with a first relapse suggestive of MS.<sup>9,10</sup> We realize this is a small series, but if the results are confirmed in larger studies, the 14-3-3 assay could be included in the decision-making algorithms to define patients who may benefit from early treatment at the time of the first relapse.

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