BRIEF REPORT

Clearance of 14-3-3 Protein from Cerebrospinal Fluid Heralds the Resolution of Bacterial Meningitis

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The 14-3-3 protein, a cerebrospinal fluid (CSF) marker of neuronal damage that was recently adopted for the diagnosis of Creutzfeldt-Jakob disease, is also found in the CSF of patients with a variety of neurological disorders. We prospectively studied 12 consecutive patients with purulent bacterial meningitis and found that 14-3-3 protein was detected in all patients at admission to the hospital. All patients who recovered cleared 14-3-3 protein from the CSF before discharge from the hospital (this was the first CSF marker to clear), whereas those who died never cleared the protein.

The disease severity and evolution of bacterial meningitis are estimated by serial measurements of inflammatory and metabolic markers in CSF [1], but no markers of neuronal damage have ever been adopted in patients with this condition [2, 3]. In the case of Creutzfeldt-Jacob disease (CJD), a rapidly progressive form of dementia belonging to the transmissible spongiform encephalopathies, the presence of 14-3-3 protein in the CSF has been found to have significant in vivo diagnostic properties in the appropriate clinical setting [4]. The presence of 14-3-3 in CSF is thought to result from neuronal disruption and the leakage of brain proteins into the CSF, and this protein was found in CSF specimens obtained from patients with different neurological pathologies whose common feature was the presence of some degree of neuronal loss [5, 6]. Although its lack of specificity clearly implies substantial limitations in the use of 14-3-3 protein as a specific CJD marker, its value as an indicator of neurological damage could be used to monitor the evolution of different neurological disorders with etiologies that can be otherwise established. On the basis of this hypothesis, we prospectively evaluated the presence of 14-3-3 protein in CSF specimens obtained from patients with bacterial purulent meningitis.

Patients and methods. All consecutive patients with a microbiologically documented diagnosis of purulent bacterial meningitis who were admitted to our department during the period of January through December 1998 were enrolled in the study and observed until discharge or death. Analyses of CSF samples included cell count, biochemistry determination, total protein level, direct microscopy on stained smears, testing for bacterial antigens (latex test), and culturing on aerobic media. Informed consent was obtained from patients or relatives.

CSF aliquots of 100 μL were mixed with 7 volumes of ice-cold methanol, kept at −20°C for 2 h, and then centrifuged at 20,800 *g* for 30 min. The pellet was dissolved in 40 μL of sample buffer (3% SDS, 3% β-mercaptoethanol, 2 m*M* EDTA, 10% glycerol, and 62.5 m*M* Tris [pH, 6.8]) and boiled for 5 min. For each sample, 10 μL (the equivalent of 25 μL of CSF), 5 μL, and 1.25 μL of sample buffer/well were loaded onto a 13% polyacrylamide gel and transferred to polyvinyliden difluoride membranes (Immobilon P; Millipore). Membranes were incubated with anti–14-3-3 β polyclonal rabbit IgG (Santa Cruz Biotechnology) at a 1:500 dilution and revealed with anti-rabbit horseradish peroxidase IgG (Amersham) at a 1:3000 dilution. The blots were developed using an enhanced chemiluminescent system (Amersham). Densitometric values for each sample were obtained with a computer-assisted laser scanner (GS-710 Calibrated Imaging Densitometry; BioRad), after correction for background. The total amount of 14-3-3 protein as quantified from each diluted and undiluted CSF sample was expressed in arbitrary units. Control CSF specimens included samples obtained from 3 patients with benign intracranial hypertension and from 2 patients with normotensive hydrocephalus.

Results. Twelve adult patients with microbiologically proved purulent bacterial meningitis were studied. The etiologic agents grown from CSF samples obtained at admission to the hospital were *Streptococcus pneumoniae* ($n = 5$), *Neisseria meningitidis* ($n = 4$), *Staphylococcus aureus* ($n = 2$), and *Pseudomonas aeruginosa* ($n = 1$). In 3 patients, meningitis developed after cranial or spinal surgery (meningitis due to *S. aureus* in 2 patients and *P. aeruginosa* in 1).

Empirical antibiotic treatment was administered at admission to the hospital and was subsequently modified (if neces-

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sary) once information on the etiology and in vitro susceptibility of the isolates became available. Three patients with pneumococcal meningitis and 1 patient with meningococcal meningitis died; all other patients recovered, with no apparent major neurological sequelae at 3–6 months of follow-up.

Respective CSF parameters (±SD) on admission for survivors and those who died were as follows: mean WBC count, 6051 ± 4241 and 4625 ± 4498 cells/μL (*P,* NS); mean level of total proteins, 275 ± 205 and 532 ± 450 mg/dL (*P,* NS); and mean glucose level, 26 ± 22 and 42 ± 32 mg/dL (*P,* NS).

The results concerning the determination of 14-3-3 protein in the CSF are presented in figure 1. All patients tested positive at admission to the hospital. The 8 patients who survived (patients 1–8) cleared 14-3-3 protein from their CSF, whereas those who died never cleared the protein.

In 6 of 8 survivors (patients 1–6), a straightforward benign course was observed, as was also indicated by the number of CSF samples obtained from these subjects (2–8 lumbar punctures performed over 5–14 days) and by the progressive downgrading tendency of all CSF markers. In these 6 patients, the first CSF sample that tested negative for 14-3-3 protein was followed only by further negative samples (or coincided with the last sample taken from the patient). The other 2 survivors (patients 7 and 8) had an intermittent and more prolonged course, with 16 and 8 lumbar punctures done over 27 and 21 days, respectively. In these 2 patients, 14-3-3 protein behaved accordingly: before final clearance was definitively established, 14-3-3 protein showed some intermittence that was concordant with both the transient clinical recurrences and the fluctuation of CSF inflammatory markers. It is noteworthy that, in these patients, bacterial meningitis (due to *S. aureus* in patient 7 and *P. aeruginosa* in patient 8) developed after cranial surgery, which is known to predispose patients toward disease recurrence [3].

Regarding the quantitation of 14-3-3 protein at admission, no significant differences were found between survivors and those who died (mean ± SD, 0.93 ± 0.66 vs. 0.81 ± 0.49 densitometric units; *P,* NS). A tendency toward lower 14-3-3 levels before clearance was seen in all survivors, whereas, in 2 of 4

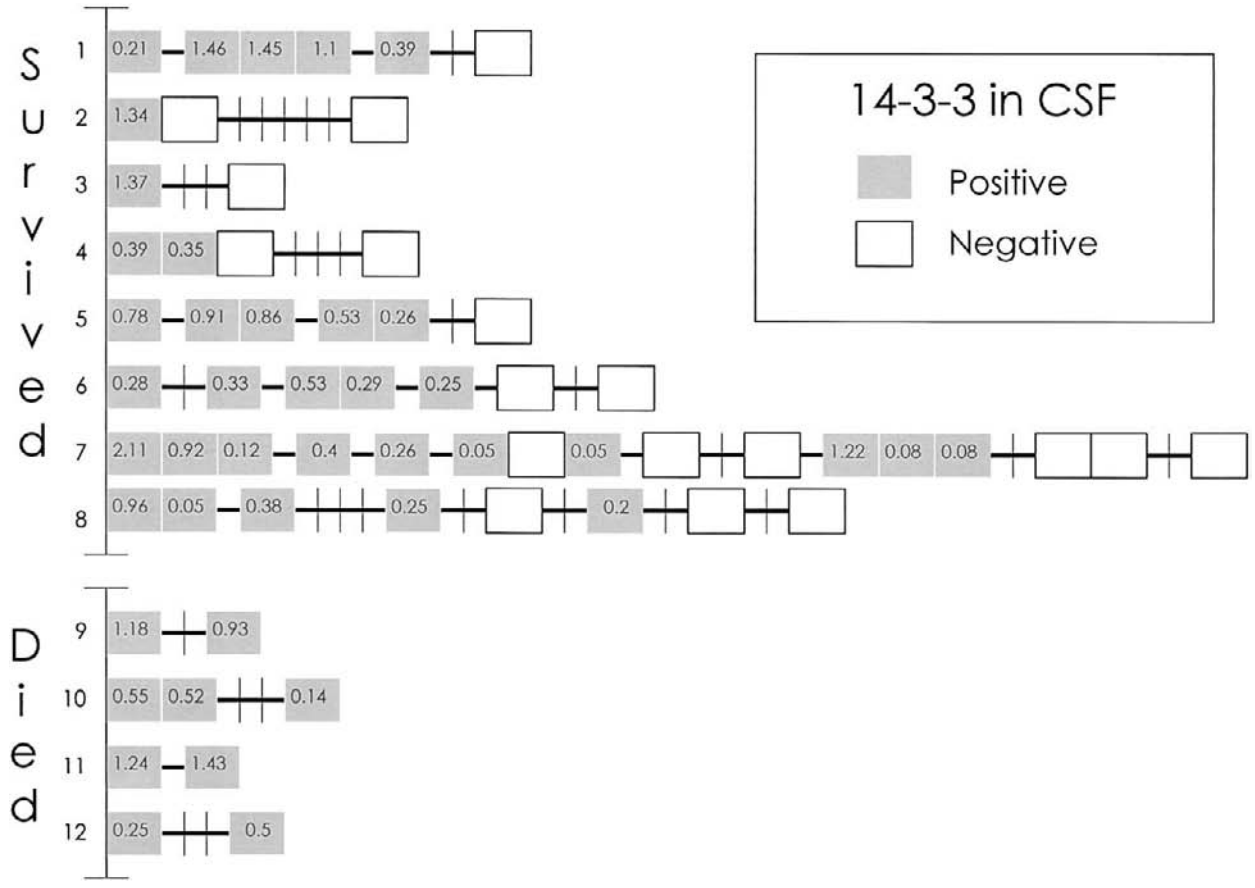

Figure 1. Results of the determination of 14-3-3 protein level in the CSF specimens obtained from patients with bacterial meningitis. *Top,* Patients who survived. *Bottom,* Patients who died. For each patient, the lumbar punctures performed are represented as squares; gray squares correspond to CSF samples that tested positive by the 14-3-3 assay (densitometric quantitation is indicated in each square), and white squares correspond to samples that tested negative. The horizontal bars represent the number of days between 2 subsequent lumbar punctures; where no bars are interposed between 2 consecutive squares, the corresponding lumbar punctures were performed on 2 consecutive days.

patients who died, the protein level was found to have increased before death.

The values of CSF parameters recorded at final clearance of 14-3-3 protein (for the survivors, patients 1–8) or at the last sampling before death (patients 9–12) are reported in table 1. In no cases did WBC counts and protein levels decrease to normal levels at the time of definitive 14-3-3 clearance in survivors.

Discussion. In 12 patients with purulent bacterial meningitis, in addition to the commonly tested CSF parameters, we serially measured the CSF concentration of 14-3-3 protein, the recently adopted marker for the in vivo diagnosis of CJD [4]. The most desirable property for a test to be used in a clinical setting is that it provides an unambiguous distinction between health and disease. According to the results we recorded, 14-3-3 protein seems to actually meet the major requirements for a neuropathologic marker in this setting.

All of the patients whom we investigated had a positive test result at admission, and all of those who recovered cleared 14-3-3 protein from the CSF before being discharged from the hospital. Conversely, the patients who died never cleared 14-3-3 protein from the CSF.

A property of 14-3-3 protein that has never been clinically tested is the time that it requires to be cleared from the CSF. This has relevant bearings in this setting, because, in being used to monitor the evolution of a reversible condition, a marker of tissue damage must quickly clear once the damaging process has subsided. As was seen in the patients who recovered, and especially in the 2 patients who had undergone neurosurgery and who had an intermittent disappearance of 14-3-3 protein before final clearance was established, the clearance time of this neuropathologic marker from the CSF may be as short as 1 day, thus providing substantial real-time information about the neurolytic activity of the ongoing infection.

It is noteworthy that, among the 8 patients who recovered, a longer persistence, as well as intermittence (positive-negative-positive), of the 14-3-3 signal before final clearance was seen in the 2 subjects who had an atypical form of meningitis (post-neurosurgical meningitis) and the longest clinical course (such as the slowest recovery, as testified by the higher number of lumbar punctures done during a longer course), thus confirming the concordance of this marker with the disease course. In the other 6 survivors, who had a more rapid and straightforward benign course, a quicker 14-3-3 protein clearance from CSF was recorded.

In the patients who recovered, clearance of 14-3-3 protein occurred before the other CSF parameters returned to normal values, which thus suggests that the clearance of 14-3-3 protein may actually be the earliest CSF parameter able to provide a distinct reliable and favorable prognostic sign. Accordingly, in pathophysiological terms, these findings also suggest that neuronal loss may actually cease before inflammatory marker levels decrease to normal levels.

Table 1. CSF findings (14-3-3 protein level and conventional major parameters) recorded at the time of final 14-3-3 protein clearance in those who recovered from bacterial meningitis (patients 1–8) and at the last determination before death in those who died (patients 9–12).

Outcome, patient	14-3-3 protein, DU[a]	WBC count, cells/μL[b]	Protein level, mg/dL[c]	Glucose level, mg/dL[d]
Survived				
1	Negative	48	54.4	37
2	Negative	3600	163.5	29
3	Negative	38	68.1	32
4	Negative	300	62.3	49
5	Negative	19	54.9	48
6	Negative	1250	166.8	58
7	Negative	36	168.1	49
8	Negative	115	127.7	59
Died				
9	0.92	640	150	38
10	0.14	11	111	62
11	1.43	1000	288.8	83
12	0.5	371	360	33

[a] CSF is normally negative for 14-3-3 protein. DU, densitometric units.
[b] Normal range, 1–10/μL.
[c] Normal range, <45 mg/dL.
[d] Normal range, 50–80 mg/dL.

Although techniques allowing the quantitation of 14-3-3 protein in the CSF are being standardized [7, 8], in the studies performed so far, the search for 14-3-3 protein in the CSF has been made only for diagnostic purposes, and, as a consequence, the 14-3-3 protein assay has so far been standardized only for a categorical response (positive or negative). Nevertheless, on the basis of the results shown here, the straightforward "on-off" signal provided by the test in monitoring neuronal damage in purulent bacterial meningitis supports the consideration of the 14-3-3 protein assay as a neuropathologic marker to be added to the panel of conventional CSF parameters.

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