

Assessment of damage to the central nervous system by determination of S-100 protein in the cerebrospinal fluid

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SUMMARY S-100 protein was determined by Particle Counting ImmunoAssay in the CSF of patients with various neurological disorders. With a limit of sensitivity of 2.5 µg/l this brain-specific protein was detected only in samples from patients with acute damage of the central nervous system, particularly in compression of the spinal cord by tumour, ischaemic disorders, subarachnoid bleeding and haematoma, and viral or suspected viral infections. Our results support the assumption that S-100 is a reliable index of central nervous system damage and that changes in its concentration could have a prognostic value.

S-100 protein is an acidic protein of molecular weight 21,000¹ found essentially in the nervous system of vertebrates.² This protein is called "S-100" because of its solubility in 100% saturated ammonium sulphate at neutral pH. A characteristic of S-100 is its structural changes caused by calcium ions.³ S-100 is, in fact, a family of proteins⁴⁻⁸ shown by complement fixation and cross-immunoelectrophoresis⁹ to be antigenically different. Two main types have been described.^{6,7} S-100a containing two different subunits (α and β subunits) and S100b, two identical subunits (β subunits).

S-100, the function of which is still unknown, is located mainly in the astrocytes¹⁰⁻¹⁹ but has also been detected in the interstitial cells of the pineal gland,¹⁶ in satellite cells of the adrenal medulla and the superior cervical ganglion,²⁰ in the pituicytes and the stellate cells of the pituitary gland,^{21,22} in melanocytes,²³ in cells with morphological features of Langerhans cells in the skin,²³ in malignant melanomas,^{24,25} and in acoustic neurinomas, sometimes in large amounts.²⁶

The purpose of the present work was to test the

hypothesis that S-100 is released into the cerebrospinal fluid (CSF) by lesions of the central nervous system (CNS) and that its determination can be useful in assessing the extent of brain damage.

Materials and methods

Patients The CSF was collected for routine analysis from patients of the Department of Neurology, Cliniques Saint-Luc, Brussels by spinal puncture. After centrifugation 0.5 to 2 ml aliquots were kept frozen at -20°C. The clinical records of these patients were reviewed retrospectively. Patients with unconfirmed diagnoses were excluded from the study. The control group comprised non-neurological patients suffering from minor neurosis but devoid of clinical signs of neurological disorders. Electroencephalography and computed tomography were normal, as well as CSF analyses comprising total protein content (below or equal to 0.4 g/l), cell count (below or equal to 5/cmm) and agar gel electrophoresis.

Purification of S-100 S-100 was isolated from bovine brains obtained at the abattoir immediately after death of the animal and purified as described by Steward.⁴ The last step was an elution from a DEAE-cellulose column (1.5 × 15 cm) by a linear gradient of 250 ml of potassium phosphate buffer, (0.01 M, pH 7.2, containing 1 mM EDTA, and 1 mM 2-mercaptoethanol) in 0.05 M NaCl, and 250 ml in 0.6 M NaCl. The main fraction was collected, extensively dialysed and stored lyophilised at -20°C.

Acrylamide gel electrophoresis A single band was visible in slab gel containing sodium dodecyl sulphate and in disc-gel acrylamide electrophoresis at pH 8.1 (fig 1). Addition of

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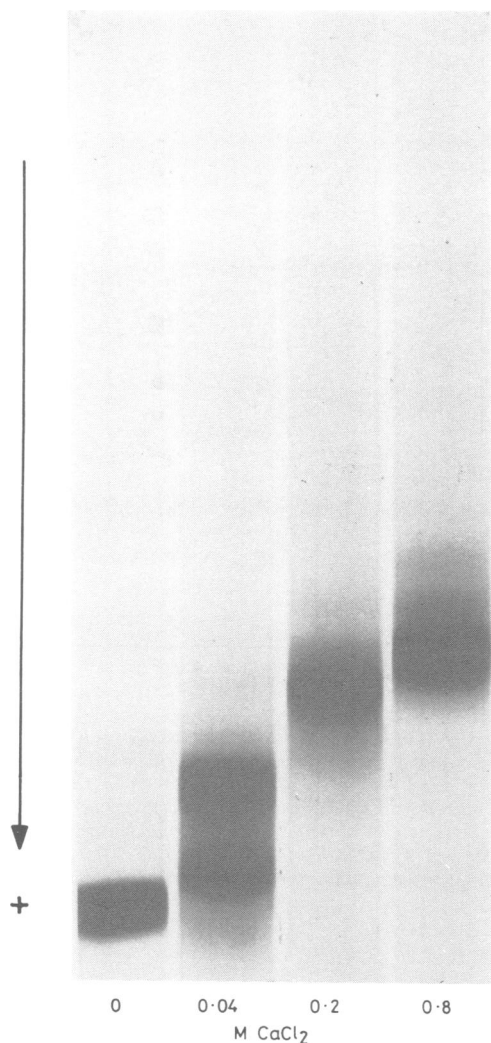


Fig 1 Disc-gel electrophoresis of 25 μ g of S-100 in 7.5% polyacrylamide gels in tris-glycine buffer, pH 8.1. From left to right: in absence of CaCl_2 and in presence of 0.04, 0.2 and 0.8 M CaCl_2 . Electrophoresis was run until the marker dye (bromophenol blue) migrated 7.5 cm.

Ca^{++} to samples, gel and buffers slowed down the mobility of S-100 in disc-gel electrophoresis (fig 1) because of the structural changes³ of the protein.

Anti-S-100 antiserum Four rabbits were injected twice a month with a methylated bovine serum albumin S-100 conjugate.²⁷ Bovine serum albumin (bSA) was from Calbiochem, La Jolla, California. After three injections, blood (50 ml) was collected one week after each injection. The antiserum was absorbed on bSA and on a pool of sera of 1,000 blood donors. The absorbing antigens were coupled

to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden).²⁸ After precipitation by half-saturated ammonium sulphate the IgG of the antiserum was isolated by DEAE-cellulose chromatography in 0.1 M tris (hydroxymethyl) methylamine-HCl, pH 8.5, and then digested by pepsin (Sigma Chemical Co, St-Louis, Mo.) as previously described.²⁹ The F(ab')_2 fragments were purified on an Ultrogel AcA 4-4 column (2.5×100 cm) (LKB, Bromma, Sweden) in 1 M NaCl buffered by 0.01 M phosphate buffer, pH 7.2. Single precipitin lines were obtained in agarose gel double immunodiffusion with bovine and human brain aqueous extracts; both lines fused completely. No reaction occurred with liver extracts. This antiserum was used in an immunofluorescence study of primary cultures of brain cells from newborn rats.³⁰ Specific fluorescence was seen in the cytoplasm and nuclei of astrocytes. Cultures of fibroblasts from meninges were not stained.

Immunoassay of S-100 S-100 levels in the CSF were determined by Particle Counting ImmunoAssay (PACIA) (Technicon International Division, Geneva, Switzerland). This now fully automated technique is based on latex agglutination. Particles coated with antibodies were agglutinated by the antigen to be determined.³¹ The use of F(ab')_2 fragments rather than whole antibody molecules avoided non-specific agglutination or inhibition of agglutination by proteins interacting with the Fc region of IgG.³² Residual interferences by protein-protein interaction were avoided by the use of a detergent (Tween 20, Technicon) in a final concentration of 3.3 ml/l. Agglutination was measured by counting the residual unagglutinated particles in an optical cell counter whose electronics have been modified to ignore aggregates. The anti-S-100 F(ab')_2 fragments were coupled to carboxylated polystyrene particles of 0.8 μ diameter (100 g/l; Estapor K 150, batch No 314, Rhône-Poulenc, Courbevoie, France) as previously described.³³ To 50 μ l (100 g/l) carbodiimide-activated latex was added 300 μ g F(ab')_2 . Such a preparation which was sufficient for 300 assays was stored at -20°C in small aliquots. The assays were calibrated with purified S-100, serially diluted in 0.1 M glycine buffer, pH 9.2, containing 9 g/l of NaCl (GBS), 10 g/l bSA and made 5 mM in CaCl_2 . Into the reaction tube were injected sequentially and automatically 30 μ l each of the sample, of the GBS containing 5 mM CaCl_2 , 60 g/l polyethylene glycol and 10 ml/l Tween 20, and of the F(ab')_2 -coated particles 200 times diluted in GBS containing 2.5 ml/l Ficoll (Pharmacia). Incubation time was 35 minutes.

Results and Discussion

THE S-100 IMMUNOASSAY

A plot of peak heights or concentration of free particles vs log of S-100 concentration formed a decreasing sigmoid curve from 2.5 to 250 μ g/l (fig 2). For greater accuracy samples containing more than 50 μ g/l were serially diluted. The most important factor which affected the immunoassay was the presence or absence of Ca^{++} (fig 3), as already observed in immunoradiometric assay³⁴ but not by electroimmunodiffusion or by microcomplement fixation

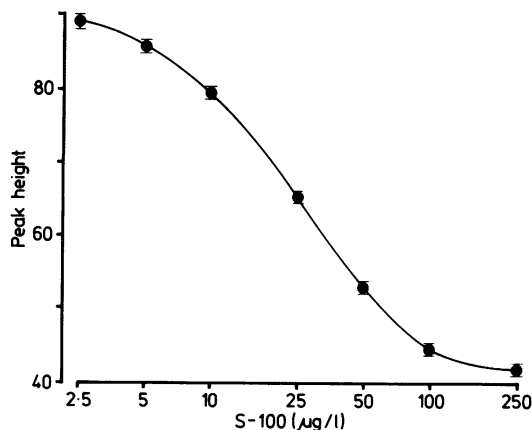


Fig 2 Calibration curve for the assay of S-100 by PACIA in the presence of 5 mM CaCl_2 . The peak heights are directly proportional to the number of non-agglutinated particles. Standard errors are calculated on five determinations within one day.

tests.⁹ Since 5 mM of CaCl_2 appeared optimum, assays were performed at this concentration.

ANALYTICAL RECOVERY

S-100 was assayed in 22 CSF samples mixed with an equal volume of either GBS containing 5 mM CaCl_2 and 10 g/l BSA or the same medium containing 50 µg/l of S-100. Analytical recovery was 95% with a coefficient of variation (CV) of 13%.

PRECISION

The intra-assay precision was studied in three CSF samples containing 7.7, 11 and 19 µg/l of S-100, the assays being repeated 10 times on the same day. The CV were respectively 12.7, 4.7 and 4.4%. The inter-assay precision was studied on samples at three concentrations of S-100, 4.5, 9.9 and 24 µg/l, the assays being repeated once each day for 8 days. The CV were respectively 16.9, 7 and 6.7%.

S-100 LEVEL IN THE CSF, AN INDEX OF CNS DAMAGE

Seventy-one samples out of 397 (18%) contained detectable amounts of S-100 (> 2.5 µg/l). The CSF samples from 29 non-neurological patients and from patients with sciatica ($N = 20$), cervical arthrosis myelopathy ($N = 17$), lumbar stenosis ($N = 9$), degenerative disorders of the CNS ($N = 12$) and seizures ($N = 3$) were apparently devoid of S-100.

High levels of S-100 and a high proportion of positive samples were found in four types of neurological diseases: compression of the spinal cord by tumour, ischaemic disorders, subarachnoid bleeding

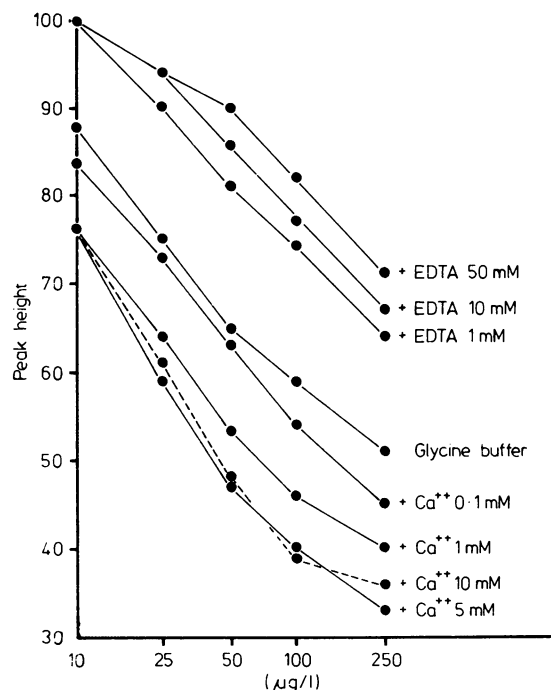


Fig 3 Effect of Ca^{++} on the immunoassay of S-100. In this experiment, 25 µl of various concentrations of S-100 diluted in GBS containing either EDTA or CaCl_2 were vortex-mixed with 25 µl of the F(ab)'_2 coated particles for 30 min at room temperature. The GBS ionic strength was kept constant by addition of sodium chloride. After mixing and appropriate dilution, the agglutination was measured as usual.

and haematoma, and viral or suspected viral infections (fig 4). All these disorders have in common acute damage of the CNS.

Samples with high concentration of S-100 were retested at three or four dilutions. All of the results were distributed in parallel to the calibration curve. Therefore one may assume that the immunoreactive material was actually S-100 and that fragments or aggregates of S-100 presumably did not interfere in the assay.

The patients of the stroke group were not representative of this type of disease because lumbar puncture is usually reserved for those with difficult diagnosis such as thrombosis of basilar and spinal arteries, which irrigate a relatively small region of the brain. In this case, less S-100 should be released than in case of large hemispheric infarction. One has also to take into account that the CSF is often collected a few days after the stroke with the possibility, that

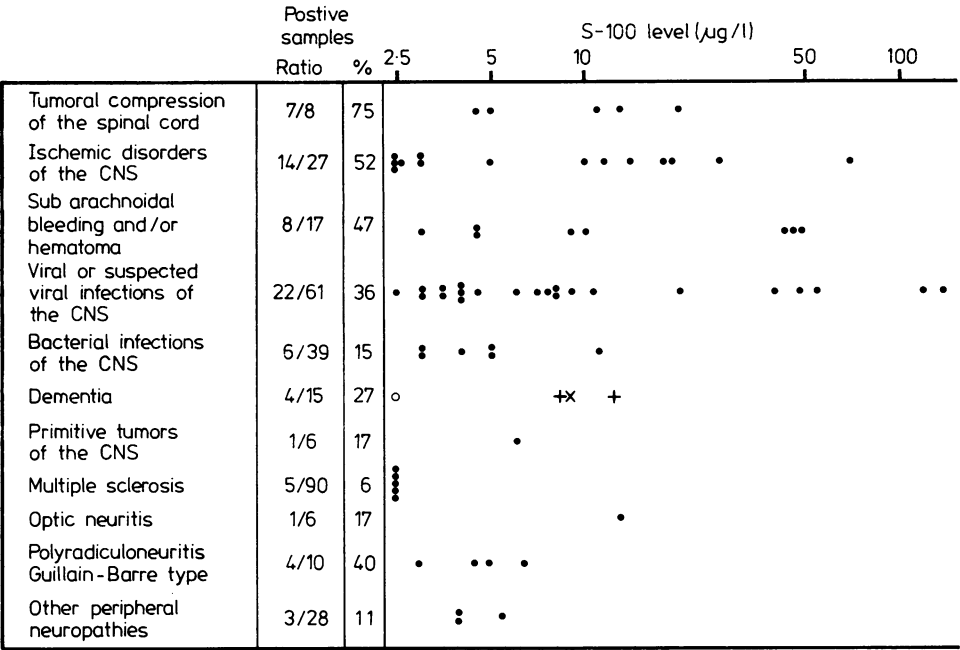


Fig 4 Determination of S-100 in the CSF of patients with various neurological disorders. The detection limit was 2.5 µg/l (+: Creutzfeldt-Jakob disease; x: multi-infarct dementia; O: pre-senile dementia).

S-100 released by the necrotic tissue has been eliminated.

Interestingly, in viral or suspected viral infections of the CNS, S-100 was found not only in patients with herpetic encephalitis and transverse myelitis, both of which extensively injure the CNS, but also in some patients with benign lymphocytic meningitis (5 cases

out of 17). This suggests that the infectious process affected not only the meninges but also the nervous tissue. It should be noted that significant amounts of S-100 were found in the CSF of four patients out of 10 with Guillain-Barré polyradiculoneuritis, whereas only three patients out of 28 with other peripheral neuropathies were positive. High levels of S-100 in

Table CSF levels of S-100 and the patients' outcome

Patients	Diagnoses	Samples	S-100 levels (µg/l)	Interval between onset of the disease and sampling (days)	Outcome
RD	Herpetic encephalitis	1st	48	7	died
		2nd	< 2.5	13	
		3rd	6	25	
		4th	8	32	
GV	Herpetic encephalitis	1st	10.5	3	partial recovery
		2nd	8.8	7	
		3rd	3	9	
		4th	< 2.5	22	
RM	Herpetic encephalitis	1st	< 2.5	3	complete recovery
		2nd	3.5	4	
		3rd	2.5	12	
GM	Subacute transverse myelitis	1st	20	28	no recovery
		2nd	54	42	
ED	Subacute transverse myelitis	1st	120	26	no recovery
		2nd	140	42	
RC	Acute transverse myelitis	1st	40	9	complete recovery
		2nd	< 2.5	23	
JB	Stroke	1st	70	2	partial recovery
		2nd	18	4	

two subsequent samples collected at one week interval from a patient with rapidly progressive Creutzfeld-Jakob disease emphasised the potential importance of the S-100 level as an index of CNS injury.

In most cases of multiple sclerosis we have failed to detect S-100. Out of 90 samples five were only slightly positive. These results contrasted with those reported by Michetti et al^{35,36} who found by complement fixation S-100 concentrations above 6 µg/l in the CSF of 70% of their patients with acute relapse of multiple sclerosis. This finding is rather surprising as recent immunohistochemical studies failed to detect S-100 in oligodendrocytes^{11,13} which are selectively destroyed with the myelin sheath by multiple sclerosis. Whereas our 90 patients had low levels of S-100 one patient out of six with optic neuritis had a high level of S-100. One may therefore wonder whether this particular case was related or not to multiple sclerosis.

PROGNOSTIC VALUE OF S-100 LEVELS

In three patients with herpetic encephalitis we observed that the prognosis of the disease was related to the level of S-100 in the first sample of CSF taken during the acute phase of the disease (table). In our study of transverse myelitis, also restricted to three patients, the poor prognosis was associated more with increased or persistent high levels of S-100 than to the absolute value in the first sample. It should be noted that in a patient who recovered from stroke the S-100 level dropped from 70 µg/l to 18 µg/l in only two days.

In conclusion, our results support the assumption that S-100 in the CSF is a reliable index of CNS injury^{35,36} and our study of a few patients suggest that the S-100 level and its changes could have a prognostic value.

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