CONCISE REPORT

Raised serum S100B protein levels in neuropsychiatric lupus

C B Schenatto, R M Xavier, M Bredemeier, L V C Portela, A B L Tort, T L Dedavid e Silva, D O Souza, J C T Brenol

Ann Rheum Dis 2006;**65**:829–831. doi: 10.1136/ard.2005.048330

Objective: To test serum S100B protein levels in patients with and without neuropsychiatric systemic lupus erythematosus (NPSLE) and controls.

Methods: 87 patients with SLE, 23 with and 64 without neuropsychiatric involvement, and 25 control subjects were prospectively evaluated. NPSLE diagnosis was made according to the American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. Serum S100B protein levels were determined with a luminescence immunoassay. Statistical analysis was performed using Mann-Whitney and Kruskal-Wallis tests.

Results: Among the patients with NPSLE, 9 presented psychosis; 4, cranial neuropathy; 3, cerebrovascular disease; 1, seizures; 1, chorea; 1, peripheral polyneuropathy; 1, multiplex mononeuropathy; 3, dementia. Serum concentrations of S100B protein were significantly higher in patients with NPSLE (median 0.164 ng/ml, interquartile range 0.113–0.332) than in non-NPSLE patients (0.062 ng/ml, 0.026–0.109) and controls (0.088 ng/ml, 0.013–0.124) ($p < 0.001$). Patients with anti-dsDNA antibodies had higher S100B protein levels ($p = 0.001$). No significant associations were found of lupus activity (among non-NPSLE cases), antiphospholipid antibodies, and reduced complement levels with S100B concentration.

Conclusions: Serum S100B protein level is raised in NPSLE, reflecting continuing neurological damage. The association of anti-dsDNA antibodies with higher S100B protein concentration deserves further study.

Involvement of the nervous system in systemic lupus erythematosus (SLE) is an important source of morbidity and mortality¹ and has a prevalence as high as 83% in some series of patients.^{2,3} Neuropsychiatric systemic lupus erythematosus (NPSLE) has a broad spectrum of manifestations, including psychiatric disorders and neurological syndromes of the central, peripheral, and autonomic nervous system.⁴ The pathogenesis of NPSLE remains poorly understood, but mechanisms like immune complex mediated vasculopathy, neurone reactive autoantibodies, thrombosis associated with antiphospholipid antibodies, and cytokine enhanced autoimmunity have been implicated.⁵

The diagnosis of NPSLE is difficult to establish because of the frequent association with other conditions and the diversity of psychiatric and neurological manifestations.⁴ Moreover, there are no specific diagnostic tests or markers of disease activity for NPSLE.

S100B is a 21 kDa calcium binding protein, produced and released predominantly by astrocytes,^{6,7} which has been investigated as a biochemical marker of central nervous system injury. Raised levels have been reported in several psychiatric and neurological diseases with different aetiologies (traumatic, vascular, and degenerative).⁷ Our group has

described raised S100B serum levels in a small sample of patients with SLE, especially in those with severe NPSLE.⁸ The present study was performed to further investigate S100B serum levels in a larger group of patients with SLE.

PATIENTS AND METHODS

Subjects

Eighty nine patients with SLE according to the 1982 American College of Rheumatology criteria,⁹ and 25 controls were prospectively evaluated in an incident case-control study. Patients seen at the rheumatology service of the Hospital de Clínicas de Porto Alegre (HCPA) were recruited between January 2000 and December 2002. Patients with overlapping syndromes, acute or chronic central nervous system infections, sepsis, metabolic or electrolyte disturbances, delirium or psychosis induced by drugs, malignancies, or hypertensive encephalopathy were excluded. Controls were healthy blood donors recruited at the blood bank centre of HCPA. All patients (or responsible relatives) and controls signed written informed consent. The study was approved by the research ethics committee of the HCPA.

Clinical evaluation

Patients were interviewed and examined by the same researcher (CBS) according to a protocol specifically designed for the study. Disease activity was measured using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI).¹⁰ The Beck Depression Inventory (BDI)¹¹ and Mini-Mental State Examination (MMSE)¹² were used to evaluate depression and cognitive function, respectively. The diagnosis of neuropsychiatric syndromes was made as suggested by the American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes.⁴ Patients with psychiatric disturbances were further evaluated by a staff psychiatrist in order to certify the diagnosis. Cases of NPSLE were classified as acute (recent onset or worsening of neuropsychiatric manifestations) or chronic (stable neuropsychiatric manifestations for more than 3 months).

Serum S100B protein levels

The samples from patients with NPSLE were collected within 1 week of the index clinical event. Serum was obtained by centrifugation at 3000 *g* for 5 minutes and kept frozen at -70°C until analysis. Levels of S100B protein in serum were determined in duplicate using a luminescence immunoassay (LIAMat; BYK-Sangtec, Broma, Sweden), as previously described.¹³

Abbreviations: BDI, Beck Depression Inventory; CI, confidence interval; HCPA, Hospital de Clínicas de Porto Alegre; IQR, interquartile range; MMSE, Mini-Mental State Examination; NPSLE, neuropsychiatric systemic lupus erythematosus; SLE, systemic lupus erythematosus; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index

Table 1 Clinical, demographic, and laboratory features of the 87 patients with SLE

Features	NPSLE		Non-NPSLE	
	Acute (n = 18)	Chronic (n = 5)	Active (n = 37)	Inactive (n = 27)
Female, No (%)	17 (94)	5 (100)	30 (81)	26 (96)
White subjects, No (%)	13 (72)	3 (60)	29 (78)	21 (78)
Age (years), mean (SD)	30.2 (9.8)	34.6 (7.2)	39.8 (13.7)	42.7 (9.7)
Disease duration (years), median (IQR)	6.5 (2–14)	4 (1–7)	8 (4–13)	9 (7–11)
SLEDAI score, median (IQR)	12 (9–16)	0 (0–14)	5 (3–6)	0 (0–0)
MMSE score, median (IQR)	25.5 (22–28)	25 (24–27)	27 (25–28)	27 (26–29)
BDI score, median (IQR)	22.75 (12–37)	5 (4–7)	10 (8–23)	13 (10–20)
Anti-dsDNA antibodies, No (%)	5 (28)	1 (20)	5 (14)	0 (0)
Antiphospholipid antibodies, No (%)	5 (28)	0 (0)	8 (22)	3 (11)
Reduced complement levels, No (%)	10 (56)	1 (20)	23 (62)	1 (4)
Current prednisone use, No (%)	17 (94)	5 (100)	32 (86)	14 (52)
Current immunosuppressive drugs, No (%)	15 (83)	4 (80)	26 (70)	3 (11)

Serology

Antinuclear antibodies were tested by the indirect immunofluorescence method using HEp-2 cells as substrate and considered positive in titres $\geq 1/80$. Anti-dsDNA was determined by indirect immunofluorescence on *Crithidia luciliae*. Serum complement protein levels (C3, C4) were measured by laser nephelometry. Anticardiolipin antibodies (IgG and IgM isotypes) were determined by direct binding enzyme linked immunosorbent assay (ELISA). The screening of lupus anticoagulant was made with the test for the activated partial thromboplastin time and with the dilute Russell's viper venom test.

Statistical analysis

Data were analysed using EPI-INFO version 6.04d and SPSS for Windows, version 11.0. Categorical variables were presented as numbers and proportions, variables with normal distribution were presented as mean and standard deviation (SD), and non-normal quantitative variables were presented as median and interquartile range (IQR). The Kruskal-Wallis

Figure 1 Comparison of S100B protein levels in different groups of patients and controls. Circles represent outliers. Kruskal-Wallis, $p < 0.001$. Acute NPSLE group statistically different from controls and non-NPSLE groups ($p < 0.05$) by Dunn's test.

test (followed by Dunn's test) and Mann-Whitney test were applied to perform between-group comparisons of S100B protein levels. A two-tailed p value ≤ 0.05 was considered significant.

Receiver operating characteristic curves were used to test the ability of the S100B protein level to differentiate patients with and without NPSLE. Ninety five per cent confidence intervals (95% CIs) for the areas under the curves were calculated. Standard measures of test validity, including sensitivity and specificity values (along with the 95% CI), were also estimated.

RESULTS

Table 1 describes the 87 patients. The controls comprised 23 women and two men, with a mean (SD) age of 38.3 (12.3) years.

Among the patients with NPSLE, nine presented psychosis; four, cranial neuropathy; three, cerebrovascular disease; one, seizures; one, chorea; one, peripheral polyneuropathy; one, multiplex mononeuropathy; three, dementia.

Patients with NPSLE had significantly higher S100B levels than non-NPSLE patients and controls (table 2, fig 1). The contrast between patients with and without NPSLE was seen even if patients with acute cerebrovascular disease and convulsive patients, which are a priori expected to have raised

Table 2 Comparison of serum levels of S100B protein (ng/ml) according to clinical and laboratory abnormalities in patients with SLE

Abnormalities	S100B protein levels		p Value*
	Median	IQR	
NPSLE			
Yes (n = 23)	0.164	0.113–0.332	<0.001
No (n = 64)	0.062	0.026–0.109	
SLEDAI score			
>0 (n = 57)	0.101	0.044–0.166	0.075
=0 (n = 30)	0.063	0.021–0.097	
MMSE score			
≤24 (n = 17)	0.100	0.056–0.194	0.200
>24 (n = 70)	0.070	0.027–0.138	
BDI score			
≥10 (n = 56)	0.076	0.026–0.152	0.076
<10 (n = 31)	0.072	0.039–0.150	
Antiphospholipid antibodies			
Yes (n = 16)	0.109	0.035–0.152	0.532
No (n = 71)	0.071	0.028–0.150	
Anti-dsDNA antibodies			
Yes (n = 11)	0.203	0.135–0.434	0.001
No (n = 76)	0.069	0.028–0.125	
Reduced complement levels			
Yes (n = 35)	0.072	0.039–0.164	0.388
No (n = 52)	0.076	0.026–0.131	
Nephritis			
Yes (n = 37)	0.072	0.045–0.138	0.780
No (n = 50)	0.079	0.026–0.164	

*Mann-Whitney test.

S100B titres, were excluded from the analysis (median, IQR: 0.135, 0.061–0.254 ν 0.062, 0.026–0.109, respectively; $p < 0.001$). There was no significant difference in serum levels between acute and chronic NPSLE (fig 1), although there were only five patients in the latter group.

Patients with anti-dsDNA antibodies had higher serum S100B protein levels (table 2). This difference was particularly marked among patients with NPSLE (median, IQR: 0.383, 0.203–0.496 in anti-dsDNA positive patients ν 0.127, 0.061–0.187 in anti-dsDNA negative patients; $p = 0.009$). The association between anti-dsDNA and S100B levels was independent of the presence of nephritis and was significant ($p < 0.05$) when patients with and without nephritis were analysed separately.

The ability of S100B protein levels to differentiate patients with and without NPSLE was tested using receiver operating characteristic curves. S100B protein levels showed good discriminatory capacity for NPSLE (area under the curve, 95 % CI 0.77, 0.65 to 0.89) and an even better capacity for acute NPSLE (0.82, 0.71 to 0.93). The cut off point ≥ 0.125 ng/ml would provide a sensitivity of 73.9% (95% CI 51.3 to 88.9) and a specificity of 79.8% (69.7 to 87.3) for NPSLE. For acute NPSLE, the same cut off value would provide a sensitivity of 77.8% (51.9 to 92.6) and a specificity of 79.8% (69.7 to 87.3).

DISCUSSION

In a preliminary study, we observed higher serum levels of S100B in patients with SLE than in controls.⁸ In the present report, S100B levels were significantly higher in patients with defined neuropsychiatric syndromes than in controls and non-NPSLE patients. The results also suggest that S100B protein levels may be a useful test in the diagnosis of NPSLE, particularly in acute forms of this syndrome.

In acute brain damage conditions (trauma, ischaemia, and haemorrhage), as well as in chronic neurodegenerative diseases (Alzheimer's disease and Down's syndrome), there is an intensive astrocytic activation called reactive gliosis. There is an increase in the synthesis and secretion of astrocytic proteins, such as the S100B.⁷ In SLE, several mechanisms, like the coexistence of alterations in the normal blood-brain-barrier, the serum antibodies directed against brain tissue, and the presence of perivascular gliosis¹⁴ might explain the observed rise in serum S100B.

We have found an association between increased S100B levels and the presence of anti-dsDNA antibodies. There is no clear explanation for this finding, but a new hypothesis for the pathogenesis of the central nervous system injury in NPSLE may offer some clue to understanding this issue. DeGiorgio *et al* have reported cross reactivity between anti-dsDNA antibodies and an amino acid sequence present in murine and human *N*-methyl-D-aspartate subtype of glutamate receptors.¹⁵ These cross reactive antibodies might induce neuronal apoptosis in vivo and in vitro,¹⁵ possibly leading to secondary gliosis and increased S100B release.

Although we do not expect that S100B will become a specific test for the differential diagnosis of neuropsychiatric lupus, our data indicate its potential usefulness as a non-specific marker of central nervous injury in these patients. Future studies with larger samples and with prospective designs will be necessary to examine such issues.

Authors' affiliations

C B Schenatto, R M Xavier, M Bredemeier, T L Dedavid e Silva, J C T Brenol, Division of Rheumatology, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

L V C Portela, A B L Tort, D O Souza, Biochemistry Department, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

Correspondence to: Professor R M Xavier, Serviço de Reumatologia, Faculdade de Medicina, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2350 – sala 645 A, CEP: 90035-903, Porto Alegre, RS, Brazil; rmxavier@hcpa.ufrgs.br

Accepted 26 October 2005

REFERENCES

- Rosner S, Ginzler EM, Diamond HS, Weiner M, Schlesinger M, Fries JF, *et al*. A multicenter study of outcome in systemic lupus erythematosus. II. Causes of death. *Arthritis Rheum* 1982;**25**:612–17.
- Appenzeller S, Costallat LT. Primary central nervous system involvement in systemic lupus erythematosus. *Revista Brasileira de Reumatologia* 2003;**43**:20–5.
- Brey RL, Holliday SL, Saklad AR, Navarrete MG, Hermosillo-Romo D, Stallworth CL, *et al*. Neuropsychiatric syndromes in lupus: prevalence using standardized definitions. *Neurology* 2002;**58**:1214–20.
- ACR. The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. *Arthritis Rheum* 1999;**42**:599–608.
- Bruyn GA. Controversies in lupus: nervous system involvement. *Ann Rheum Dis* 1995;**54**:159–67.
- Heizmann CW. Ca²⁺-binding S100 proteins in the central nervous system. *Neurochem Res* 1999;**24**:1097–100.
- Rothermundt M, Peters M, Prehn JH, Arolt V. S100B in brain damage and neurodegeneration. *Microsc Res Tech* 2003;**60**:614–32.
- Portela LV, Brenol JC, Walz R, Bianchin M, Tort AB, Canabarro UP, *et al*. Serum S100B levels in patients with lupus erythematosus: preliminary observation. *Clin Diagn Lab Immunol* 2002;**9**:164–6.
- Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997;**40**:1725.
- Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 1992;**35**:630–40.
- Gorenstein C, Andrade L. Validation of a Portuguese version of the Beck Depression Inventory and the State-Trait Anxiety Inventory in Brazilian subjects. *Braz J Med Biol Res* 1996;**29**:453–7.
- Bertolucci PH, Brucki SM, Campacci SR, Juliano Y. [The Mini-Mental State Examination in a general population: impact of educational status]. *Arq Neuropsiquiatr* 1994;**52**:1–7.
- Lara DR, Gama CS, Belmonte-de-Abreu P, Portela LV, Goncalves CA, Fonseca M, *et al*. Increased serum S100B protein in schizophrenia: a study in medication-free patients. *J Psychiatr Res* 2001;**35**:11–14.
- Zvaifler NJ, Bluestein HG. The pathogenesis of central nervous system manifestations of systemic lupus erythematosus. *Arthritis Rheum* 1982;**25**:862–6.
- DeGiorgio LA, Konstantinov KN, Lee SC, Hardin JA, Volpe BT, Diamond B. A subset of lupus anti-DNA antibodies cross-reacts with the NR2 glutamate receptor in systemic lupus erythematosus. *Nat Med* 2001;**7**:1189–93.