Utility of Serum S100B Protein for Identification of Central Nervous System Involvement in Systemic Lupus Erythematosus

HILDA FRAGOSO-LOYO, JAVIER CABIEDES, YEMIL ATISHA-FREGOSO, LUIS LLORENTE, and JORGE SÁNCHEZ-GUERRERO

ABSTRACT. ***Objective.*** To evaluate utility of S100B protein in serum as a marker of central nervous system involvement in systemic lupus erythematosus (SLE).

Methods. Forty patients with SLE, hospitalized because of central neuropsychiatric (cNP) manifestations (n = 36) and peripheral NP manifestations (pNP, n = 4) were studied. Patients were evaluated at hospitalization and 6 months later, including a serum and cerebrospinal fluid (CSF) sample. As controls, 4 SLE patients with septic meningitis (SLEsm), 13 surgical SLE patients (SLE surgical), 14 patients with nonautoimmune diseases, and 4 patients with primary NP syndromes were included. Serum and CSF S100B protein levels were determined by ELISA.

Results. At baseline, serum levels of S100B protein did not differ across SLE groups. Using an arbitrary cutoff value, positive S100B levels in serum were observed in 7 (19%), 6 (46%), and 1 patient from the cNPSLE, SLE surgical, and SLEsm groups, respectively. S100B protein levels in cNPSLE and SLE surgical patients were similar. In CSF, S100B protein levels did not differ among SLE groups, except in patients with SLEsm. Paired serum/CSF samples were obtained in 23 patients with cNPSLE at 6 months after the acute event. Overall, levels of S100B protein in serum did not change despite the decrease observed in CSF (p = 0.004). The correlation coefficient of serum and CSF S100B protein levels among all the SLE patients at baseline was poor (r = 0.23).

Conclusion. Serum levels of S100B protein do not differentiate SLE patients with and those without central neurological manifestations. (J Rheumatol First Release August 15 2010; doi:10.3899/jrheum.100148)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS CENTRAL NERVOUS SYSTEM S100B PROTEIN

Despite the available information regarding the presence of autoantibodies and other inflammatory molecules in the cerebrospinal fluid (CSF) of patients with neuropsychiatric systemic lupus erythematosus (NPSLE), the mechanisms of entry and the timepoint in the course of disease at which a breach in the blood-brain barrier (BBB) occurs remain unknown. It has been our tenet that the breach of the BBB could be a sporadic but recurring phenomenon[1,2]. Thus, autoantibodies and other inflammatory molecules not secreted intrathechally may gain access into the central nervous system (CNS) through an alteration of the BBB. For this reason, it would be of utmost importance to have a biomarker that can reliably reflect BBB damage for monitoring CNS involvement in SLE.

From the Department of Immunology and Rheumatology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, México, DF, México.

H. Fragoso-Loyo, MD; J. Cabiedes, PhD; Y. Atisha-Fragoso, MD; L. Llorente, MD; J. Sánchez-Guerrero, MD, MS.

Address correspondence to Dr. J. Sanchez-Guerrero, Department of Immunology and Rheumatology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Vasco de Quiroga 15, Tlalpan, 14000, México, DF, México. E-mail: jsanchezguerrero7@gmail.com

Accepted for publication June 16, 2010.

One possible marker of brain damage is calcium-binding protein S100B, predominantly expressed and secreted by astrocytes in vertebrate brain[3]. S100B belongs to a family of calcium-binding proteins implicated in intracellular and extracellular regulatory activities[4,5]. Intracellularly, it exhibits regulatory effects on cell growth, differentiation, cell shape, and energy metabolism. Extracellularly, S100B stimulates neuronal survival, differentiation, astrocytic proliferation, neuronal death via apoptosis; it also stimulates or inhibits activity of inflammatory cells. Moreover, elevation in serum S100B protein has been reported to reflect injury to the brain and increased permeability of the BBB. Although it is commonly associated with severe brain injury such as contusions, swelling, and diffuse axonal injury, it is also found in nontraumatic forms such as trisomy 21, Alzheimer's disease, and brain tumors[6,7].

In SLE, serum S100B protein levels were found to be significantly higher in NPSLE than in non-NPSLE patients and healthy controls, showing good discriminatory capacity for NPSLE and an even better capacity for acute NPSLE[8]. Serum and CSF levels were raised in SLE patients with organic brain syndrome, seizure, cerebral vascular accident, and psychosis, but not among patients with headache and

neuropathy in comparison to controls. These results imply that S100B protein might be useful in the evaluation and diagnosis of NPSLE, particularly the acute and diffuse forms[9].

Previously, we defined the cytokine and chemokine profile in CSF[1], as well as the behavior and association of serum and CSF autoantibodies in patients with NPSLE[2]. The aim of the present study was to assess the utility of S100B protein levels in serum as a marker of brain damage and their correlation with CSF levels, in patients with SLE and central neuropsychiatric (cNP) manifestations during the acute event and 6 months later when the neurological manifestations were clinically inactive.

MATERIALS AND METHODS

Forty patients with SLE according to the American College of Rheumatology (ACR) criteria[10] hospitalized between February 2003 and June 2005 for cNP manifestations (n = 36) and peripheral NP (pNP) manifestations (n = 4) were included. All patients were evaluated by the study rheumatologists and neurologists at hospitalization and 6 months later using a standardized protocol, including disease activity assessment by SLE Disease Activity Index 2000 (SLEDAI-2K)[11]. At hospitalization, information on sociodemographic data and SLE characteristics (i.e., duration of disease) was collected. Serum and CSF samples were obtained in all patients at hospitalization and 6 months later. NP manifestations were classified using the ACR nomenclature for NP lupus syndromes[12], and patients were categorized as cNPSLE group: seizure disorders, n = 15, severe refractory headache 8, acute confusional state 7, cerebrovascular disease 4, psychosis 1, and pseudotumor cerebri 1; and pNPSLE group: multiplex mononeuritis 3, and polyneuropathy 1. NP manifestations were attributed to SLE given that there were no exclusion factors for it[12], and no patient had any of the minor NP events reported with comparable frequency in the general population[13].

As controls, serum and CSF samples were also obtained from 4 SLE patients with septic meningitis (SLEsm group), 13 SLE patients without history of NP manifestations who underwent an elective surgery (SLE surgical group), 14 patients with neither autoimmune diseases nor NP manifestations who also underwent elective surgery (nonautoimmune group), and 4 patients with primary NP syndromes.

In all cases, serum and CSF samples were obtained during clinical assessment on arrival at the hospital. Serum was collected, and CSF was centrifuged at 12,000 g. Serum and CSF supernatant were immediately frozen (< 30 min) at –86°C until assayed for determination of S100B protein levels. Serum and CSF S100B protein levels were determined with an ELISA assay kit according to the manufacturer's recommendations (BioVendor Laboratory Medicine Inc., Candler, NC, USA). This assay has a sensitivity of 0.05–20 μg/l.

Statistical methods. We compared levels of serum and CSF S100B protein among SLE patients with and without central neurological manifestations, patients with nonautoimmune diseases, and patients with primary NP syndromes.

Since S100B protein levels in humans have yet to be established and because S100B cutoff values for the presence and extent of brain injury remain unknown, we arbitrarily defined as positive those values above 3 SD from the mean values observed in serum and CSF, respectively, among the patients with nonautoimmune diseases.

Categorical variables were compared using chi-square or Fisher's exact test. Continuous variables were analyzed using one-way analysis of variance across study groups. Because S100B protein levels were not normally distributed, they were log-transformed, and differences between groups were tested using Student t-test. Paired-samples analyses were performed using Wilcoxon signed-ranks test. Since it was planned in advance to analyze the levels of the S100B protein between the cNPSLE and each one of the other study groups, correction for multiple comparisons was not considered.

In addition, we calculated sensitivity, specificity, positive and negative predictive values, and accuracy of positive S100B protein levels in serum for the presence of central neurological manifestations using 2 × 2 tables. Correlation between serum and CSF S100B protein levels was estimated using Spearman correlation coefficient. P value was set at < 0.05, 2-tailed. Analysis was performed using the Stata 10.0 computer program.

RESULTS

Population characteristics. Mean age of study patients was cNPSLE 30.9 years ± 11.9, pNPSLE 24.0 ± 7.07, SLE surgical 39.4 ± 9.2, SLEsm 25.5 ± 9.7, primary NP syndromes 24.3 ± 10.5 years, and nonautoimmune diseases 37.2 ± 13.9 years (p = 0.70). Disease activity was moderate/severe in patients with cNPSLE, pNPSLE, and SLEsm in comparison to the SLE surgical group (p = 0.002). Also, duration of lupus was shorter in the former groups (p = 0.03). Details of disease characteristics among the study groups are shown in Table 1.

Among patients with cNPSLE, in 22 (52%) no associated factors for the NP manifestations were present, and in 20 (48%) concurrent, non-exclusion factors were identified[12]. Among the pNPSLE patients there were no associated factors for NP manifestations.

Patients in the SLE surgical group underwent the following elective surgical procedures: hysterectomy (4), renal biopsy (2), hip/knee arthroplasty (2), laminectomy (1), Hallux valgus (1), splenectomy (1), myomectomy (1), and Tenchkoff catheter collocation (1).

The primary NP syndromes were: epilepsy in 1, optic neuritis in 1, primary CNS vasculitis in 1, delirium in 1, and Devic's syndrome in one patient.

Nonautoimmune disease patients underwent elective surgery because of the following conditions: bone marrow donors (4), hysterectomy (3), Tenchkoff catheter collocation (1), hydrocele (2), lower limb amputation due to diabetes mellitus type II (1), saphenectomy (2), circumcision (1), and inguinal hernioplasty (1). The microorganisms responsible for septic meningitis in the SLE patients were: *Streptococcus pneumoniae* (1), *Lysteria monocytogenes* (1), *Mycobacterium tuberculosis* (1), and *Staphylococcus sp*. (1).

Serum and CSF S100B protein levels. *Baseline*. Serum levels of S100B protein did not differ across different SLE groups. And CSF values showed no clear difference among SLE groups, except in SLEsm, where the highest levels were observed (Figure 1).

In serum, positive S100B levels were observed in 7 (19%) patients with cNPSLE manifestations, in 6 (46%) in the SLE surgical group, and in 1 patient in the SLEsm group. The levels observed in the cNPSLE and SLE surgical groups were similar (Table 2).

In CSF, positive S100B levels were detected in 7 (19%) patients with cNPSLE manifestations, in 3 (75%) in the

Table 1. Demographic and clinical characteristics of the study patients at hospitalization. Except where indicated otherwise, values are the mean ± SD.

Characteristic	cNPSLE, n = 36	pNPSLE, n = 4	SLE Surgical, n = 13	SLE sm, n = 4	Primary NP, n = 4	Nonautoimmune, n = 14	p
Age, yrs	30.9 ± 11.9	24.0 ± 7.07	39.4 ± 9.2	25.5 ± 9.7	24.3 ± 10.5	37.2 ± 13.9	0.7
Male/female	6/30	0/4	2/11	0/4	0/4	4/10	
SLEDAI-2K score	14.9 ± 9.3	15.5 ± 8.06	3.8 ± 2.9	8.8 ± 5.9	—	—	0.002
SLE duration, yrs	2.1 ± 2.6	2.2 ± 2.5	8.9 ± 6.9	2.04 ± 2.4	—	—	0.03

SLE: systemic lupus erythematosus; cNPSLE: central neuropsychiatric SLE; pNPSLE: peripheral NPSLE; Primary NP: Nonrheumatic neurological diseases. SLEDAI-2K: SLE Disease Activity Index 2000 update; SLEsm: SLE septic meningitis.

SLEsm group, and in 1 patient in the primary neurological group. The levels of S100B protein among positive patients were similar across the 3 groups (Table 2).

The cNPSLE manifestations between S100B-positive and S100B-negative patients were similar. Interestingly, no patient with pNPSLE manifestations tested positive for S100B protein, in either serum or CSF.

At 6 months. Paired serum and CSF samples were obtained in 23 patients with cNPSLE manifestations at 6 months after acute event. Overall, levels of S100B protein in serum were unchanged, but in CSF a significant decrease was observed ($p = 0.004$).

Three of 4 patients who tested positive in serum at baseline turned negative at 6 months; also, all 7 patients who tested positive in CSF initially became negative ($p = 0.009$). In addition, among the positive patients at baseline, a major decrease in the levels of S100B protein was observed in both serum and CSF ($p = 0.07$, and $p = 0.02$, respectively; Table 3).

Utility of S100B protein levels in serum as a marker of CNS involvement in SLE. Serum S100B protein levels did not differentiate SLE patients with and those without central neurological manifestations. A serum S100B protein level of 3 SD above the mean levels in nonautoimmune patients showed a sensitivity of 20%, specificity 65%, positive predictive value 57%, negative predictive value 26%, and accuracy 33%, for differentiating patients with central neurological manifestations.

The correlation coefficient of S100B protein levels in serum and CSF among all the SLE patients at baseline was $r = 0.23$ ($p = 0.08$).

DISCUSSION

We assessed utility of S100B protein levels in serum as a marker of brain damage and its correlation with CSF levels in 4 different groups of patients with SLE, in patients with nonautoimmune diseases, and in patients with primary NP syndromes. Overall, serum S100B protein levels did not appear to be a useful marker to discriminate SLE patients with and without CNS involvement, and its correlation with CSF levels was weak.

We studied a well defined population of patients with cNPSLE, pNPSLE, SLE without NPSLE, SLEsm, patients with non-autoimmune diseases, patients with no history of NP manifestations, and patients with primary NP disorders. Previously, we reported the profiles of cytokines, chemokines, and autoantibodies in this population[1,2].

Measurement of S100B protein in serum and CSF was done by ELISA using a commercial kit, and following the manufacturer's recommendations. The mean CSF/serum ratio of S100B protein levels in the cNPSLE group and in nonautoimmune patients was 13.1 and 8.4, respectively, which is between the range described in other studies[14,15,16]. Thus we consider that the S100B protein levels were measured correctly.

Because normal values of S100B protein in humans have yet to be established and S100B cutoff values for the presence and extent of brain injury remain undefined, we analyzed our results in 2 different ways: (1) we compared the levels of serum S100B protein among SLE patients with and without cNP manifestations; and (2) we defined as a positive S100B protein test those levels > 3 SD above the mean value found in the serum of nonautoimmune patients.

Therefore, considering the characteristics of patients included, the accuracy of measuring S100B protein levels, and the analyses of the results, we are confident about our study conclusions.

Two studies have assessed S100B protein in SLE[8,9]. In the first, serum S100B protein levels were significantly higher in patients with NPSLE compared with non-NPSLE patients and healthy controls, with good discriminatory capacity for NPSLE and better capacity for acute NPSLE[8]. The second study showed that serum and CSF levels were raised in SLE patients with organic brain syndrome, seizure, cerebral vascular accident, and psychosis, but not among patients with headache and neuropathy in comparison to controls[9]. These results imply that S100B protein might be useful in the evaluation and diagnosis of NPSLE, particularly the acute and diffuse forms. These findings must be analyzed carefully because their mean CSF/serum S100B ratios from their NPSLE and control groups, 1.43 and 1.1, respectively, are far too low versus those commonly reported[14,15,16].

In contrast with these studies[8,9], we observed that S100B protein levels in serum did not differ between patients with and those without cNPSLE at the onset of acute manifestations. And no difference was observed in CSF, except in SLEsm patients, where the highest levels were observed.

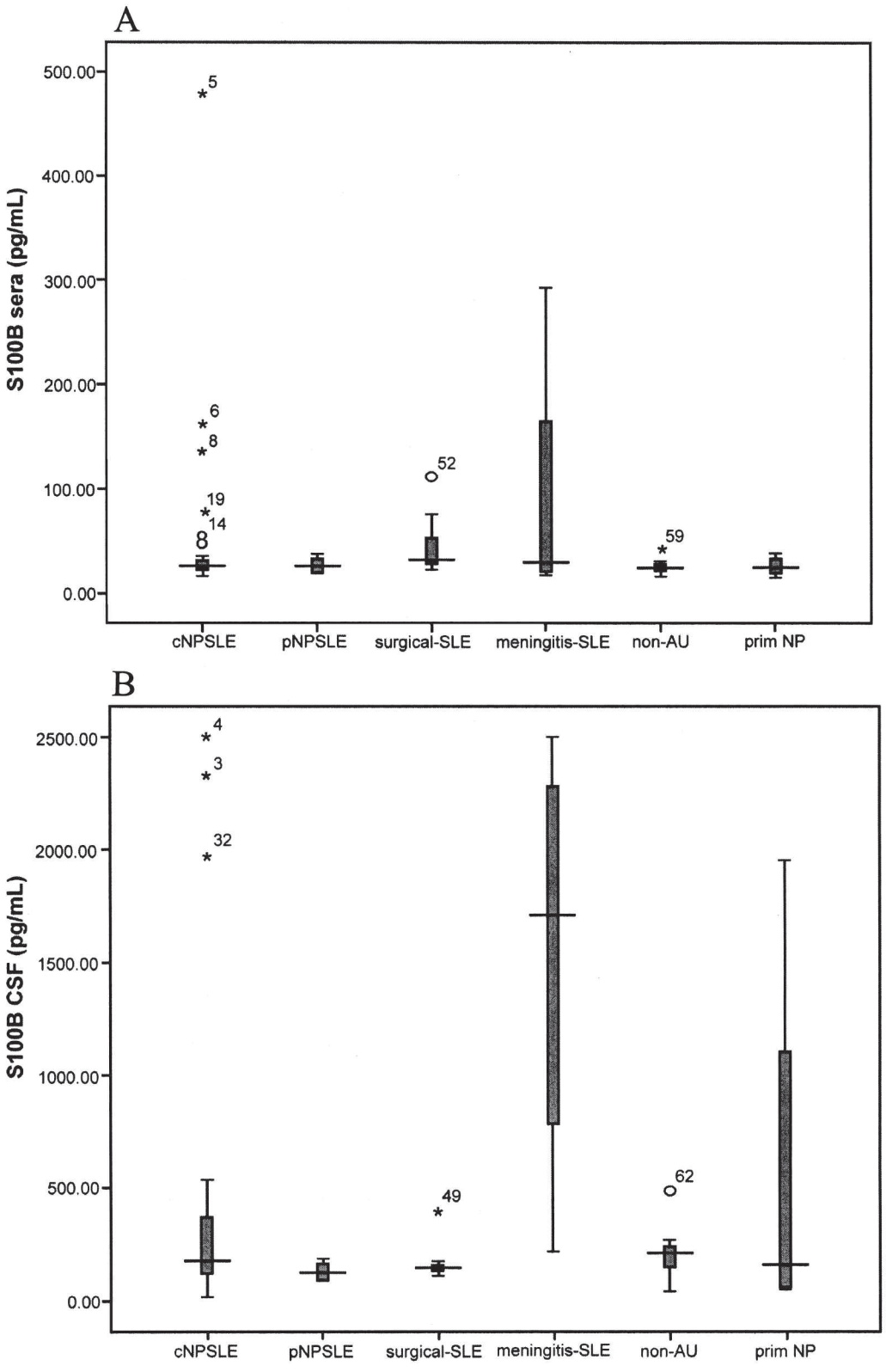

Figure 1. Comparison of serum (A) and CSF (B) S100B protein levels in patients with SLE and controls. Circles and asterisks represent outliers. cNPSLE: central neuropsychiatric SLE; pNPSLE: peripheral NPSLE; non-AU: nonautoimmune; prim NP: primary NP.

Table 2. Prevalence and levels of S100B protein in serum and cerebrospinal fluid (CSF) from NPSLE patients and controls. Values are expressed as median (min-max).

	cNPSLE, n = 36	pNPSLE, n = 4	SLE Surgical, n = 13	SLEsm, n = 4	Primary NP, n = 4	Nonautoimmune, n = 14
Serum						
S100B levels*	25.9 (16.3–479.1)	25.8 (20.3–37.9)	32.1 (22.6–111.7)	29.8 (17.3–293)	24.9 (16.4–38.7)	24.4 (16.3–42.4)
S100B-positive (%)	7 (19)	0	6 (46)	1 (25)	0	0
S100B levels**	77.0 (48.0–479.1)	0	53.2 (48.4–111.7)	293	0	0
CSF						
S100B levels*	177 (17.1–2500)	121.3 (86.7–182.4)	149.7 (108.7–393.2)	1706.8 (215.3–2500)	156.2 (46.6–1952)	208.9 (37.1–485.9)
S100B-positive (%)	7 (19)	0	0	3 (75)	1 (25)	0
S100B levels**	2325.7 (535.4–2500)	0	0	2060.8 (1352.8–2500)	1952	0

* All patients studied. ** Positive patients with levels ≥ 3 SD above the mean levels in nonautoimmune patients. SLE: systemic lupus erythematosus; cNPSLE: central neuropsychiatric SLE; pNPSLE: peripheral neuropsychiatric SLE; Primary NP: nonrheumatic neurological diseases; SLEsm: SLE septic meningitis.

Table 3. Prevalence and levels in serum and cerebrospinal fluid (CSF) from cNPSLE patients, as determined at baseline and 6 months. Values are expressed sa median (min-max).

	cNPSLE, Baseline, n = 23	cNPSLE, 6 mo, n = 23	p
Serum			
S100B levels*	24.6 (19.8–164.3)	24.6 (12.6–55.3)	0.19
S100B-positive (%)	4 (17)	1 (4)	0.35
S100B levels**	65.7 (48–164.3)	29.7 (22.8–55.3)	0.07
CSF			
SB100 levels*	265.4 (68.9–2500)	154.2 (65.5–525.8)	0.004
S100B-positive (%)	7 (30)	0	0.009
S100B levels**	2325.7 (535.4–2500)	130.2 (65.5–256.9)	0.02

* All patients studied. ** Positive patients with levels ≥ 3 SD above the mean levels in nonautoimmune patients.

Further, serum and CSF S100B protein levels between patients with cNPSLE with and without associated factors were similar (data not shown). Paired analyses of serum and CSF S100B protein levels in cNPSLE patients at onset of the acute event and 6 months later did not show a difference in serum levels, but a significant decrease was observed in CSF. In a minority of patients who had the highest levels of S100B protein in serum and/or CSF at baseline, a decrease was observed during followup, especially in CSF.

Potential limitations of our study need to be acknowledged: (1) Although a relatively large number of patients with cNPSLE manifestations were studied, we were unable to correlate S100B protein levels with specific manifestations. However, cNPSLE diagnosis did not differ between patients with high and and those with low levels of S100B protein in serum or CSF. (2) Our results apply to patients with acute NP manifestations who needed to be hospitalized for diagnosis and/or treatment. We did not study nonhospitalized patients, or patients with chronic serious manifestations, e.g., depression, seizure, or cognitive dysfunction. (3) We did not carry out in-depth screening for NP manifestations among patients without history of NP; therefore, although none of them had any of the acute and severe manifestations included among the patients with cNPSLE and pNPSLE, we cannot exclude that they had mild or subclinical NP manifestations. (4) Although at 6 months, patients with NPSLE were clinically in remission, we cannot exclude ongoing, subclinical NP activity that might have been associated with the levels of S100B protein.

Strengths of our study: (1) We assessed levels of S100B protein simultaneously in serum and in CSF, in various groups of patients with SLE, and in subjects without autoimmune disease. Thus, several groups of negative controls were included, as well as SLE patients with infectious meningitis, an inflammatory global process in the meninges unrelated to lupus. (2) The paired assessment of S100B protein in serum and CSF during the acute episode and 6 months later provides unique information about the sensitivity of this protein to detect acute cNPSLE and the sensitivity to change during the course of the manifestations. 3. All patients were assessed prospectively following a standardized evaluation, and the S100B protein determination was blinded to diagnosis and study group. Further, all patient sera and CSF samples were evaluated simultaneously to avoid intraassay variabililty.

Our findings raise an aspect of special interest. The S100B protein levels found in CSF were in all instances at pico- or nanomolar concentrations, i.e., trophic and/or reparative activity levels as opposed to micromolar concentrations, which are considered characteristic of toxic and/or degenerative activity[4,5]. This suggests that those patients with the highest S100B protein levels were the ones with significant worse CNS damage, but this was still able to be partially restored by S100B. We therefore consider that the results obtained clearly illustrate the behavior of S100B protein in central NP manifestations. Although the BBB may be somewhat affected in SLE, pico- or nanomolar concentrations of S100B protein in CSF are not reflected systemically and thus, measurement of S100B in serum is not indicative, at least in NPSLE, of BBB damage.

In summary, the apparently high S100B protein levels in CSF from some patients with cNPSLE could be normal; this is supported by the fact that the mechanism by which a noxious stimulus triggers CNS damage in these patients affects neural tissue differently even in the same NP manifestation. On the other hand, serum S100B protein levels in cNPSLE do not correlate with levels found in CSF, thus S100B levels do not seem to be a useful biomarker of CNS involvement in patients with SLE.

REFERENCES

1. Fragoso-Loyo H, Richaud-Patin Y, Orozco-Narváez A, Dávila-Maldonado L, Atisha-Fregoso Y, Llorente L, et al. Interleukin-6 and chemokines in the neuropsychiatric manifestations of systemic lupus erythematosus. Arthritis Rheum 2007;56:1242-50.
2. Fragoso-Loyo H, Cabiedes J, Orozco-Narváez A, Dávila-Maldonado L, Atisha-Fregoso Y, Diamond B, et al. Serum and cerebrospinal fluid autoantibodies in patients with neuropsychiatric lupus erythematosus. Implications for diagnosis and pathogenesis. PLoS One 2008;3:e334.
3. Marenholz I, Heizmann CW, Fritz G. S100 proteins in mouse and man: from evolution to function and pathology (including an update of the nomenclature). Biochem Biophys Res Commun 2004;322:1111-22.
4. Donato R. S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int J Biochem Cell Biol 2001;33:637-68.
5. Schäfer BW, Heizmann CW. The S100 family of EF-hand calcium-binding proteins: functions and pathology. Trends Biochem Sci 1996;21:134-40.
6. Griffin WS, Stanley LC, Ling C, White L, MacLeod V, Perrot LJ, et al. Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. Proc Natl Acad Sci USA 1989;86:7611-5.
7. Sen J, Belli A. S100B in neuropathologic states: the CRP of the brain? J Neurosci Res 2007;85:1373-80.
8. Schenatto CB, Xavier RM, Bredemeier M, Portela LV, Tort AB, Dedavid e Silva TL, et al. Raised serum S100B protein levels in nueropsychiatric lupus. Ann Rheum Dis 2006;65:829-31.
9. Yang XY, Lin J, Lu XY, Zhao XY. Expression of S100B protein levels in serum and cerebrospinal fluid with different forms of neuropsychiatric systemic lupus erythematosus. Clin Rheumatol 2008;27:353-7.
10. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.
11. Gladman DD, Ibanez D, Urowitz MB. Systemic Lupus Erythematosus Disease Activity Index 2000. J Rheumatol 2002;29:288-91.
12. ACR Ad Hoc Committee on Neuropsychiatric Lupus Nomenclature. The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. Arthritis Rheum 1999;42:599-608.
13. Ainiala H, Hietaharju A, Loukkola J, Peltola J, Korpela M, Metsänoja R, et al. Validity of the new American College of Rheumatology criteria for neuropsychiatric lupus syndromes: a population-based evaluation. Arthritis Rheum 2001;45:419-23.
14. Kleindienst A, Meissner S, Eyupoglu IY, Parsch H, Schmidt C, Buchfelder M. Dynamics of S100B release into serum and cerebrospinal fluid following acute brain injury. Acta Neurochir Suppl 2010;106:247-50.
15. Lins H, Wallesch CW, Wunderlich MT. Sequential analyses of neurobiochemical markers of cerebral damage in cerebrospinal fluid and serum in CNS infections. Acta Neurol Scand 2005;112:303-8.
16. Gonçalves CA, Leite MC, Nardin P. Biological and methodological features of the measurement of S100B, a putative marker of brain injury. Clin Biochem 2008;41:755-63.