Acta Neurol Scand 2003: 108: 424–427 DOI: 10.1046/j.1600-0404.2003.00159.x
Printed in UK. All rights reserved

ACTA NEUROLOGICA
SCANDINAVICA

Interictal serum S100B levels in chronic neurocysticercosis and idiopathic epilepsy

Portela LVC, Tort ABL, Walz R, Bianchin M, Trevisol-Bittencourt PC, Wille PR, Cardoso RC, Ishida MMI, vonWangenheim A, Grisard EC, Steindel M, Gonçalves CA, Souza DO. Interictal serum S100B levels in chronic neurocysticercosis and idiopathic epilepsy.
Acta Neurol Scand 2003: 108: 424–427. © Blackwell Munksgaard 2003.

Objective – To assess whether serum S100B levels could reflect a glial response in patients with epilepsy secondary to neurocysticercosis (NCC) and with idiopathic epilepsy. *Subjects and methods* – Serum S100B levels were measured using an immunoluminometric assay in 20 patients with focal epilepsy related to chronic NCC (NCC group), and 19 patients with focal epilepsy (EPI group), matched by epidemiological and clinical data. Epileptic patients were compared with 20 healthy controls (CON group) matched by age and sex. *Results* – No difference was observed in S100B levels among NCC, EPI and CON groups ($P > 0.39$). Serum S100B levels were not affected by antiepileptic drugs, frequency and type of seizures. Preliminarily, significantly higher levels of S100B were observed in patients with bilateral electroencephalographic (EEG) findings than in patients with unilateral and normal EEG findings ($P < 0.05$). *Conclusion* – Serum S100B is normal in patients with focal epilepsy related or not to chronic NCC.

L. V. C. Portela[1,2], A. B. L. Tort[1], R. Walz[3], M. Bianchin[3], P. C. Trevisol-Bittencourt[4], P. R. Wille[5], R. C. Cardoso[5], M. M. I. Ishida[6], A. vonWangenheim[7], E. C. Grisard[5], M. Steindel[5], C. A. Gonçalves[1], D. O. Souza[1]

[1]Departamento de Bioquímica, ICBS, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil; [2]Laboratório Municipal de Novo Hamburgo, SEMSA, Novo Hamburgo, RS, Brazil; [3]CIREP, Centro de Cirurgia de Epilepsia, Hospital de Clínicas da USP, Ribeirão Preto, SP, Brazil; [4]Hospital Universitário, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil; [5]Radiologische Gemeinschaftspraxis, Fort Malakoff Park, Mainz, Germany; [6]Departamento de Microbiologia e Parasitologia da Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil; [7]Departamento de Informática e Estatística, UFSC, Florianópolis, SC, Brazil

Key words: S100B; epilepsy; neurocysticercosis; seizures; EEG

Luis V. C. Portela, Departamento de Bioquímica, ICBS, UFRGS. Rua Ramiro Barcelos 2600, anexo. Porto Alegre, RS, Brazil. CEP: 90035-003
Tel.: +55 51 3316-5558
Fax: +55 51 3316-5540
e-mail: roska@ufrgs.br

Accepted for publication February 10, 2003

Neurocysticercosis (NCC) is an infestation of the central nervous system (CNS) by the invasive larval stages (cysticerci) of the pork tapeworm *Taenia solium* (1). It occurs after the ingestion of contaminated pork meat or of *T. solium* eggs shed in human faeces (1). Symptomatic NCC usually results from host CNS inflammatory response after parasite death, and epilepsy, focal neurological signs, and intracranial hypertension are the most common clinical manifestations (2, 3). The disease is endemic in developing countries, including Brazil (4), where it is an important cause of secondary epilepsy (2, 5).

Reactive gliosis, known as an astrocytic response to brain injury, is a prominent neuropathological feature of epileptic foci, and may play a causal role in the development and maintenance of seizure disorders (6). This astrocytic response includes altered morphology, gene expression, proliferation and cytokines release (7). Although the role of astrocytes on the pathophysiology of epilepsy secondary to several ethiologies is not completely understood, perilesional astrogliosis has been found by specific imaging studies in a considerable number of NCC patients (8), suggesting that this astrocytic reaction is chronically sustained.

The S100B is a cytokine produced and released predominantly by astrocytes at physiological and pathological conditions, which at low extracellular concentrations have neurotrophic and gliotrophic actions implicated in the development and maintenance of the CNS (9, 10). However, elevated levels of S100B in cerebrospinal fluid (CSF) and serum could indicate reactive gliosis, astrocytic

death and/or blood–brain barrier dysfunction (11), which allows to use it as a potential peripheral marker of several acute and chronic CNS diseases, including traumatic brain injury (12), brain ischemia (13) and hemorrhage (14), Alzheimer's disease (15), schizophrenia (16), and tropical spastic paraparesis (17).

Considering the potential involvement of glial cells on NCC seizures, as well as in other epilepsy etiologies, the present work was performed to investigate the interictal serum S100B levels in patients with seizures related or not to chronic NCC.

Subjects and methods

Interictal serum levels of S100B protein were measured in 39 epileptic patients: 20 with epilepsy associated to chronic NCC (NCC group) and 19 with epilepsy without NCC (EPI group). They were followed at the Policlínica Regional – SUS, Florianópolis, SC, southern Brazil, and were compared with 20 healthy blood donors (CON group) matched by age and sex (18). Epileptic patients were matched by age, sex, age of epilepsy onset (considered the age that patients started the recurrent epileptic seizure), epilepsy duration, frequency of seizures, interictal electroencephalographic (EEG) abnormalities, seizure types, and antiepileptic drug (AED) used.

Epilepsy diagnosis was performed by history, neurological examination, computer tomography (CT) and routine EEG examination (at least 30-min duration, 10 of 20 system for electrodes). None of the patients presented other neurological or psychiatric symptoms. Patients had simple partial or complex partial epilepsy, with at least one episode of secondarily generalized seizure during their life. Focal abnormalities of the interictal EEG including spikes, spikes and waves, sharp waves, were classified as focal unilateral or focal bilateral. Frequency of seizures was classified as: high (weekly to monthly seizure attacks), intermediate (between 1/month to 1/six months) or low (rarer than 1/six months).

The AED used were carbamazepine (monotherapy), or carbamazepine plus other drug (phenobarbital, valproic acid or clobazam). Serum levels of AED were not assessed in the same samples tested for S100B, but treatment compliance was considered high in the sample. No patient showed signs or symptoms suggestive of intoxication, and five were not using any AED during the study.

Diagnosis of NCC was based on clinical history, known epidemiological data, and calcified lesions identified by CT. It was assumed that the etiology of calcified lesions was NCC based first on the high prevalence of NCC in our midst (4, 19). Secondly, we grounded our diagnosis on several reports that established criteria for differentiating cysticerci from other lesions, such as tuberculosis and fungal infections (20, 21). No patient received previous treatment for fungal or tuberculosis infection of CNS. Patients with calcified lesions in which clinical and/or radiological data suggested etiologies other than NCC were not included.

At least 5 days after the last epileptic seizure, blood samples were collected without anticoagulant by venipuncture. Serum was obtained by centrifugation at $3000 \times g$ for 5 min and kept frozen at −70°C until the analysis. The local ethics committee approved this study, and written consent was obtained from all patients and controls.

Serum levels of S100B protein were determined using a sensitive luminescence assay (BYK-Sangtec; Bromma, Stockholm, Sweden) according to previously described protocol (22). Briefly, it is a monoclonal two-site immunoassay that uses an antibody covalently bound to isoluminol as tracer. The samples were measured in duplicate and those with a coefficient of variation above 10% had their measurement repeated. Comparisons of mean age among groups were performed by analysis of variance (ANOVA). Comparisons between NCC and EPI Groups concerning mean age of epilepsy onset and epilepsy duration were carried out by Student's '*t*' test. Comparisons between NCC and EPI groups related to frequency and type of seizures attacks, interictal EEG findings, and AED used were compared by Fisher's exact test. Comparisons of serum S100B levels among groups were performed by Kruskal–Wallis.

All epileptic patients (NCC and EPI) were also analyzed as a single group in order to determine if serum S100B levels were affected by interictal EEG findings (normal, unilateral alteration or bilateral alteration), AED scheme used (monotherapy or polytherapy), frequency (high, intermediate and low) and type of seizures (simple partial and complex partial).

A P value of < 0.05 was considered to be statistically significant in all analysis.

Results

Clinical data are provided in Table 1. There were no differences among the mean age of the subjects in the three groups ($P > 0.65$). When comparing both epileptic groups, no difference was observed between the mean age of epilepsy onset ($P > 0.45$), epilepsy duration ($P > 0.20$), frequency of attacks ($P > 0.50$), interictal EEG findings ($P > 0.90$),

Table 1 Clinical and demographic characteristics of groups. Age of subjects, age of epilepsy onset and epilepsy duration are expressed in mean years (±SD). There were no differences among the mean age of subjects ($P > 0.65$) of the three groups, and between the mean age of epilepsy onset ($P > 0.45$), epilepsy duration ($P > 0.20$), frequency of seizure ($P > 0.50$), interictal electroencephalographic findings ($P > 0.90$), seizure type ($P > 0.30$), and AED scheme used ($P > 0.30$) of the two epileptic groups

	Groups		
Variables	CON	EPI	NCC
Sex (M/F)	10/10	10/09	10/10
Age (years)	33.9 (±12.0)	32.7 (±9.1)	31 (±10.0)
Age of epilepsy onset (years)	NA	11.3 (±8.9)	13.6 (±9.8)
Epilepsy duration (years)	NA	21.7 (±9.2)	17.5 (±10.5)
Frequency of seizure			
Low	NA	06	04
Mean	NA	06	08
High	NA	07	08
Interictal EEG findings			
Normal	NA	09	09
Unilateral	NA	07	08
Bilateral	NA	03	03
Seizure type			
Simple partial	NA	09	07
Complex partial	NA	10	13
AED used			
None	NA	01	04
Monotherapy	NA	13	12
Polytherapy	NA	05	04

EEG, electronencephalography; AED, anti-epileptic drug; NA, Non-applicable in that group.

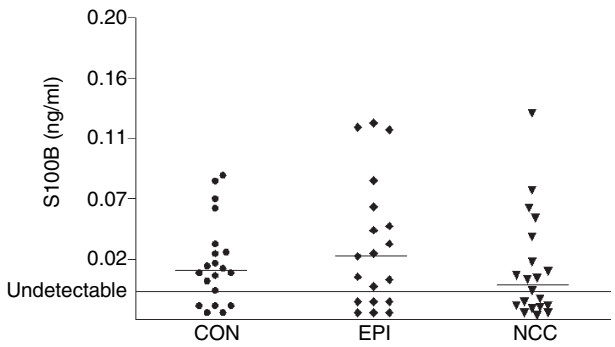

Figure 1. Serum S100B levels expressed in ng/ml of patients and controls are presented as individual values. The horizontal bars represent median value in each group. There were no statistical differences in serum S100B levels among the three groups ($P > 0.39$).

seizure type ($P > 0.30$), and AED scheme used ($P > 0.30$).

Serum S100B levels of the three groups are presented in Fig. 1. The median of serum S100B levels were 0.016 ng/ml (IQ: 0/0.034) in CON group, 0.026 ng/ml (IQ: 0/0.062) in EPI group, and 0.005 ng/ml (IQ: 0/0.036) in NCC group. No statistically significant difference was observed among the three groups ($P > 0.39$).

Analyzing serum S100B levels of all epileptic patients (NCC and EPI groups) as one group, the AED scheme used, frequency and type of seizures presented no effect on serum S100B levels ($P > 0.25$, data not shown). However, the patients with bilateral EEG findings had significantly higher levels of S100B (median: 0.055 ng/ml; IQ: 0.023/0.125) than patients with normal (median: 0.005 ng/ml; IQ: 0/0.023) and unilateral (median: 0.004 ng/ml IQ: 0/0.054) EEG findings ($P < 0.05$).

Discussion

Although there is some evidence that glial cells are involved in the pathophysiology of epileptogenesis (7), there are few studies on the correlation between S100B and epilepsy in humans. Griffin et al. (23) showed that in sections of temporal neocortex tissue from patients with intractable temporal lobe epilepsy (TLE), the number of S100B immunoreactive astrocytes was approximately threefold higher than in control patients, and these astrocytes had prominent characteristics of reactive gliosis. Steinhoff et al. (24) found elevated CSF S100B levels within the site of epileptogenic zone in patients with intractable TLE.

Besides the studies providing evidence that glial cells are involved in epileptogenesis of TLE (23, 24), some studies also pointed astrocytic involvement in other etiologies of epilepsy (6, 7). Altogether, these works suggest that reactive gliosis is an adaptive response to seizure (7), and could be related to its intensity (6). Moreover, once initiated, the astrocytic response could significantly contribute to the subsequent pathophysiologic processes (7).

Regarding peripheral S100B levels in epilepsy, Palmio et al. (25) observed normal serum and CSF S100B levels in patients with single, previous undiagnosed and untreated tonic–clonic seizures. Accordingly, in the present work, normal S100B values were found in patients with idiopathic epilepsy. A significant variation of S100B serum levels was found only in the specific subgroup of epileptic patients with bilateral EEG alterations. This preliminary result could be related to the extension of brain damage, but requires further characterization.

Concerning epilepsy related to chronic NCC, the role of astroglial cells activation on its pathophysiology has not been well established. Pradhan et al. (8) reported the presence of perilesional gliosis in 20% of NCC patients using T1-weighted magnetization transfer spin-echo magnetic resonance imaging (MRI), which was not visible on initial

MRI. It is possible that imaging methods could not be sensitive enough to detect subtler glial alterations that could be present in more patients.

In the present study, a sensitive method was employed, which detected S100B in serum of most controls and epileptic patients. We postulated that NCC would increase peripheral S100B levels, denoting secondary reactive astrogliosis. However, our result do not support this hypothesis, as no differences in S100B levels between controls and NCC patients were found.

This work does not completely discard the presence of astrogliosis in the pathophysiology of NCC, nor high content of S100B in the CNS of these patients, once the peripheral S100B assessment as a marker of glial activation is still a matter of debate. In this way, neuropathological studies would be the choice to characterize the presence or absence of glial response in NCC patients.

Conclusion

Serum S100B is normal in patients with focal epilepsy related or not to chronic NCC.

Acknowledgements

The present work was supported by CNPq and PRONEX/FINEP # 41960904. Dr Paulo Wille is a fellowship from Kottmaier-Stiftung Foundation, Germany. The authors thank Cristine Boppré for her technical support.

References

1. Duchene M, Benoudiba F, Ienecker C, Hadj-Rabia M, Caldas J, Doyon D. Neurocysticercosis. J Radiol 1999;**80**:1623–27.
2. Pal DK, Carpio A, Sander JW. Neurocysticercosis and epilepsy in developing countries. J Neurol Neurosurg Psychiatry 2000;**68**:137–43.
3. Del Brutto OH. Neurocysticercosis. Rev Neurol 1999; **29**:456–66.
4. Rigati M, Trevisol-Bittencourt PC. Cause of late-onset epilepsy in an epilepsy clinic of Santa Catarina – Southern Brazil. Arq Neuropsiquiatr 1999;**57**:787–92.
5. Carpio A, Escobar A, Hauser WA. Cysticercosis and epilepsy: a critical review. Epilepsia 1998;**39**:1025–40.
6. Khurgel M, Ivy GO. Astrocytes in kindling: relevance to epileptogenesis. Eplepsy Res 1996;**26**:163–75.
7. Jankowsky JL, Patterson PH. The role of cytokines and growth factors in seizures and sequelae. Prog Neurobiol 2001;**63**:125–49.
8. Pradhan S, Kathuria MK, Gupta RK. Perilesional gliosis and seizure outcome: a study based on magnetization transfer magnetic resonance imaging in patients with neurocysticercosis. Ann Neurol 2000;**48**:181–87.
9. Donato R. Functional roles of S100 proteins, calcium-binding proteins of the EF-hand type. Biochim Biophys Acta 1999;**1450**:191–231.
10. Gonçalves DS, Lenz G, Karl J, Gonçalves CA, Rodnight R. Extracellular S100B protein modulates ERK in astrocyte cultures. Neuroreport 2000;**11**:807–9.
11. Wong CH, Rooney SJ, Bonser RS. S100beta release in hypothermic circulatory arrest and coronary artery surgery. Ann Thorac Surg 1999;**67**:1911–14.
12. Ingebrigtsen T, Waterloo K, Jacobsen EA, Langbakk B, Romner B. Traumatic brain damage in minor head injury: relation of serum S-100 protein measurements to magnetic resonance imaging and neurobehavioural outcome. Neurosurgery 1999;**45**:468–75.
13. Wunderlich MT, Ebert AD, Kratz T, Goertler M, Jost S, Herrmann M. Early neurobehavioural outcome after stroke is related to release of neurobiochemical markers of brain damage. Stroke 1999;**30**:1190–5.
14. Gazzolo D, Vinesi P, Bartocci M et al. Elevated S100 blood level as an early indicator of intraventricular hemorrhage in preterm infants. Correlation with cerebral Doppler velocimetry. J Neurol Sci 1999;**170**:32–5.
15. Sheng JG, Mrak RE, Rovnaghi C, Kozlowska E, Van Eldik LJ, Griffin WS. Human brain S100B and S100B mRNA expression increases with age: pathogenic implications for Alzheimer's disease. Neurobiol Aging 1996;**17**:359–63.
16. Lara DR, Gama CS, Belmonte-de-Abreu P, Portela LVC et al. Increased serum S100B protein in schizophrenia: a study in medication-free patients. J Psychiatr Res 2001; **35**:11–14.
17. Walz R, Portela LVC, Tort ABL et al. Serum S100B levels in patients with HTLV-I associated myelopathy/tropical spastic paraparesis. Neurology 2000;**54**:2021–22.
18. Portela LV, Tort AB, Schaf DV et al. The serum S100B concentration is age dependent. Clin Chem 2002;**48**:950–52.
19. Trevisol-Bittencourt PC, Silva NC, Figueiredo R. Prevalence of neurocysticercosis among epileptic patients in the west of Santa Catarina – southern Brazil. Arq Neuropsiquiatr 1998;**56**:53–8.
20. Leite JP, Terra-Bustamante VC, Fernandes RMF et al. Calcified neurocysticercotic lesions and post-surgery seizure control in temporal lobe epilepsy. Neurology 2000; **55**:1485–91.
21. Rajshekhar V. Etiology and management of single small CT lesions in patients with seizures: understanding a controversy. Acta Neurol Scand 1991;**84**:465–70.
22. Missler U, Wiesmann M, Ehlermann P et al. Validation and comparison of two solid-phase immunoassays for the quantification of S-100B in human blood. Clin Chem 2000;**46**:993–6.
23. Grin WS, Yeralan O, Sheng JG et al. Overexpression of neurotrophic cytoquine S100B in human temporal lobe epilepsy. J Neurochem 1995;**65**:228–33.
24. Steinho BF, Tumani H, Otto M et al. Cisternal S100 protein and neuron-specific enolase are elevated and site-specific markers in intractable temporal lobe epilepsy. Epilepsy Res 1999;**36**:75–82.
25. Palmio J, Peltola J, Vuorinen P, Laine S, Suhonen J, Keränen T. Normal CSF neuron-specific enolase and S-100 protein levels in patients with recent non-complicated tonic-clonic seizures. J Neurol Sci 2001;**183**:27–31.