



Observational Study

## Clinical scenarios for the use of S100 $\beta$ as a marker of hepatic encephalopathy

Andrés Duarte-Rojo, Astrid Ruiz-Margáin, Ricardo U Macias-Rodriguez, Francisco Javier Cubero, José Estradas-Trujillo, Rosa Ma Muñoz-Fuentes, Aldo Torre

Andrés Duarte-Rojo, University of Arkansas for Medical Sciences, Little Rock, AR 72205, United States

Astrid Ruiz-Margáin, Ricardo U Macias-Rodriguez, José Estradas-Trujillo, Rosa Ma Muñoz-Fuentes, Aldo Torre, Department of Gastroenterology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City 14000, Mexico

Astrid Ruiz-Margáin, Ricardo U Macias-Rodriguez, Francisco Javier Cubero, Department of Internal Medicine III, University Hospital RWTH Aachen, 52074 Aachen, Germany

Francisco Javier Cubero, Department of Immunology, Complutense University School of Medicine, 28040 Madrid, Spain

**Author contributions:** Duarte-Rojo A and Ruiz-Margáin A contributed equally to this article; Duarte-Rojo A and Torre A contributed to study conception and design; Ruiz-Margáin A, Estradas-Trujillo J and Muñoz-Fuentes RM contributed to data acquisition; Duarte-Rojo A, Ruiz-Margáin A, Macias-Rodriguez RU contributed to data analysis and interpretation; Duarte-Rojo A, Ruiz-Margáin A, Macias-Rodriguez RU and Cubero FJ contributed writing the manuscript; Duarte-Rojo A, Cubero FJ and Torre A contributed to editing, reviewing and final approval of the article; and Torre A provided the funding for the project.

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**Correspondence to:** Aldo Torre, MD, MSc, Department of Gastroenterology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Vasco de Quiroga 15, Colonia Sección XVI, Tlalpan C.P., México City 14000, Mexico. [detoal@yahoo.com](mailto:detoal@yahoo.com)  
Telephone: +52-155-54870900

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### Abstract

**AIM:** To evaluate the association between serum concentrations of S100 $\beta$  in patients with cirrhosis and the presence of low grade hepatic encephalopathy (HE).

**METHODS:** This was a cross-sectional study. The population was categorized into four groups healthy subjects, cirrhosis without HE, cirrhosis with covert hepatic encephalopathy (CHE) and cirrhosis with overt HE. Kruskal-Wallis, Mann Whitney's *U* with Bonferroni adjustment Spearman correlations and area under the ROC were used as appropriate.

**RESULTS:** A total of 61 subjects were included, 46 cirrhotic patients and 15 healthy volunteers. S100 $\beta$  values were different among all groups, and differences remained significant between groups 1 and 2 ( $P < 0.001$ ), and also between groups 2 and 3 ( $P = 0.016$ ), but not between groups 3 and 4. In cirrhotic patients with HE S100 $\beta$  was higher than in

patients without HE [0.18 (0.14-0.28) ng/mL *vs* 0.11 (0.06-0.14) ng/mL,  $P < 0.001$ ]. There was a close correlation between serum concentrations of S100 $\beta$  and psychometric hepatic encephalopathy score in patients with cirrhosis without HE compared to the patients with cirrhosis with CHE ( $r = -0.413$ ,  $P = 0.019$ ). ROC curve analysis yielded  $> 0.13$  ng/mL as the best cutoff value of S100 $\beta$  for the diagnosis of HE (sensitivity 83.3%, specificity 63.6%).

**CONCLUSION:** Serum concentrations of S100 $\beta$  are higher in patients with cirrhosis than in healthy volunteers, and are further increased in the presence of hepatic encephalopathy. The results suggest that serum biomarkers such as S100 $\beta$  could help in the correct characterization of incipient stages of HE.

**Key words:** Hepatic encephalopathy; S100 $\beta$  protein; Astrocyte; Psychometric hepatic encephalopathy score; Critical flicker frequency

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**Core tip:** Hepatic encephalopathy is a complication present in 30%-80% of the patients with cirrhosis and it is associated with increased mortality, adverse clinical outcomes, and poor quality of life. An increased concern about early recognition of this complication has risen in recent years; however, no biochemical marker is available to date. In this paper we evaluated the performance of S100 $\beta$  as a biochemical marker to identify the early stages of HE, and its correlation with neuropsychometric tests.

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## INTRODUCTION

Hepatic encephalopathy (HE) reflects a spectrum of neuropsychiatric abnormalities seen in patients with liver dysfunction after exclusion of other brain diseases<sup>[1,2]</sup>. Covert hepatic encephalopathy (CHE) is the initial stage in the clinical spectrum of HE and involves subtle changes in cognitive and motor function, and electroencephalographic patterns. In contrast to patients with symptomatic HE, patients with CHE have no recognizable clinical symptoms of brain dysfunction and need to be diagnosed on the basis of neuropsychologic tests<sup>[3]</sup>. The prevalence of CHE in cirrhotics with good liver function (Child A) is 15%, while in those with advanced cirrhosis (Child B/C) it

approaches 50%<sup>[4-6]</sup>. Multiple pathways are involved in the pathophysiology of HE, however, hyperammonemia plays a central role. Astroglial swelling in HE occurs if the load of glutamine accumulating during ammonia detoxification is not compensated by a decrease in other osmolytes like myo-inositol<sup>[7]</sup>. This is followed by development of low-grade cerebral edema, which is accompanied by an increased production of reactive oxygen and nitrogen species, triggering multiple protein and RNA modifications, thereby affecting brain function<sup>[8]</sup>. Associated abnormalities in the homeostasis of cerebral neurotransmitters and blood-brain barrier (BBB) disruption have not been well characterized yet. Different molecules have been addressed as markers of brain injury, some of them being linked to astrocyte damage, but to date there is no useful serologic marker that can aid the diagnosis of HE or CHE.

S100 $\beta$  is a 10.4 kDa protein that is primarily synthesized in the brain by the endfeet processes of the astrocytes, and it belongs to a superfamily of low molecular weight acidic calcium binding proteins of the EF-hand type<sup>[9]</sup>. This protein is primarily metabolized in the kidney and excreted in the urine, its expression across ethnic groups seems to be consistent, differences between genders are not found, and it does not seem to show circadian variation<sup>[10]</sup>. Although S100 $\beta$  is not entirely specific for the central nervous system (CNS), it is present in the brain at much higher concentrations than in other tissues (80%-90% of the total pool is found within the brain), and as such, this protein can be used as an early marker of brain damage<sup>[9]</sup>.

Astrocytes are key in homeostasis regulation in the CNS, being activated after brain injury and releasing S100 $\beta$ <sup>[11]</sup>. Some studies have shown increased levels of S100 $\beta$  in patients with acute or chronic liver failure and HE which might reflect an early stage of intracerebral changes before the development of marked cerebral edema<sup>[12,13]</sup>. Moreover it has been proposed that increased serum concentrations of S100 $\beta$  could predict CHE<sup>[14]</sup>. However, evidence associating S100 $\beta$  levels and the presence of HE is scarce. The aim of this study was to evaluate the association between serum concentrations of S100 $\beta$  and the presence of low-grade HE in patients with cirrhosis.

## MATERIALS AND METHODS

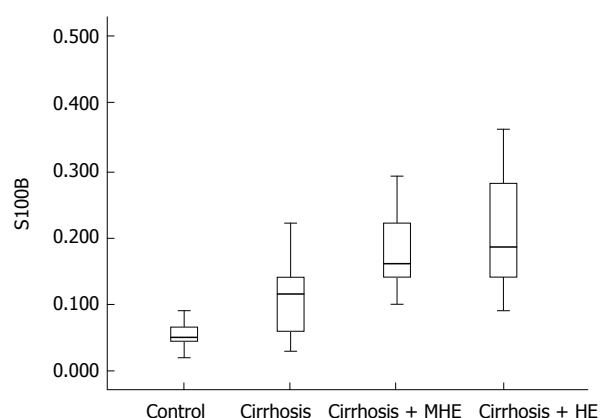
### Patients

This was a cross-sectional analytic study. Healthy volunteers and ambulatory patients with cirrhosis were invited to participate. Cirrhosis was confirmed on the basis of liver biopsy, or presence of biochemical, ultrasonographic, and/or endoscopic features of portal hypertension and liver dysfunction. High-grade HE, use of psychoactive drugs, alcohol during the 3 mo prior to the start of the study, presence of renal failure, respiratory or central nervous system (CNS) disease, cardiac failure, or severe malnutrition

**Table 1** Demographic characteristics of the study population

	Healthy subjects	C + non-HE	C + CHE	C + HE
<i>n</i>	15	22	10	14
Gender (M/F)	(8/7)	(11/11)	(6/4)	(9/5)
Age	51.5 (46-64.5)	53 (45-60)	65.5 (59-71.5)	66 (57-71)
Years of education	14 (6-19)	12 (6-16)	6 (4-9.7)	9 (6-16)
Main etiology (%)	--	HCV (34%)	HCV (40%)	HCV (66%)
MELD score	--	9.5 (8.5-11.5)	10 (7.7-15.2)	11.5 (10-17.5)
Child-Pugh (points)	--	7 (6-8)	7.5 (6-9)	10 (8-12)
Serum sodium	--	136 (129-143)	135 (126-139)	138 (122-141)
Ammonia	--	179 (113.5-224)	217 (194-250)	134 (95-277)
Total bilirubin	--	1.81 (1.11-2.48)	1.50 (1.26-2.58)	2.81 (2.09-5.94)
ALT	--	34 (28-55.2)	31 (24.5-95.5)	42.5 (32.2-65.5)
AST	--	62.5 (41-98)	49 (37.5-115)	77 (44-105.5)
Albumin	--	2.9 (2.6-3.3)	2.9 (2.6-3.2)	2.2 (2.0-2.7)
Alkaline phosphatase	--	141.5 (89.7-211)	140 (85-184)	154 (100.5-80.5)
INR	--	1.1 (1.1-1.2)	1 (0.9-1.2)	1.2 (1.1-1.7)
Creatinine	--	0.82 $\pm$ 0.19	1.11 $\pm$ 0.38	0.92 $\pm$ 0.31

Data presented as absolute frequencies, median (IQR) and mean  $\pm$  SD. C: Cirrhosis; CHE: Covert hepatic encephalopathy; HE: Overt hepatic encephalopathy.



**Figure 1** Serum concentrations of S100 $\beta$  (ng/mL) among groups. (1) Healthy subjects [0.05 (0.04-0.07)]; (2) Cirrhosis without HE [0.11 (0.06-0.14)]; (3) Cirrhosis with covert HE [0.16 (0.13-0.23)]; (4) Cirrhosis with overt HE [0.18 (0.14-0.28)]. Group 1 vs group 2,  $P < 0.001$ ; Group 2 vs group 3,  $P < 0.016$ ; Group 3 vs 4,  $P = 0.508$ . Healthy subjects vs all groups [0.14 (0.098-0.198)],  $P < 0.001$ .

were all considered exclusion criteria. History and physical examination, as well as psychometric hepatic encephalopathy score (PHES) test, and critical flicker frequency (CFF) analysis (Hepatonorm Analyzer; R&R Medi-Business Freiburg GMBH, Freiburg, Germany) were performed in all patients. Healthy volunteers did not show any neurologic abnormality on exam.

### Methods

Overt HE was diagnosed on clinical grounds and classified according to West Haven criteria (all grade I or II). Covert HE (CHE) was identified in patients without overt manifestations of HE, when a PHES score was more than 4 SD below the Mexican population norms<sup>[15]</sup>. Using above criteria, 4 groups were formed: (1) healthy subjects; (2) cirrhosis without HE; (3) cirrhosis with CHE; and (4) cirrhosis

with overt HE. Blood samples were drawn after an 8-h fasting period, and plasma collected and stored at  $-70^{\circ}\text{C}$  for further determination. S100 $\beta$  was measured using a human S100 $\beta$  enzyme-linked immunoassay (ELISA) according to manufacturer's instructions (BioVendor, Candler, United States). All tests were run in duplicate.

### Statistical analysis

All results HE are expressed in medians and inter-quartile range, except for PHES, for which we used maximum and minimum values. The Kruskal-Wallis test was used to compare differences between groups for each of the variables of interest, while the  $U$  of Mann-Whitney with Bonferroni adjustment ( $\alpha/\kappa = 0.016$ ) was used to evaluate differences between pairs of groups. Spearman test was used for bivariate correlations. ROC curve analysis was used to obtain a cutoff value for S100 $\beta$ . A  $P$  value  $< 0.05$  was considered significant. SPSS version 21.0 was used for statistical analysis (IBM, Armonk, NY).

## RESULTS

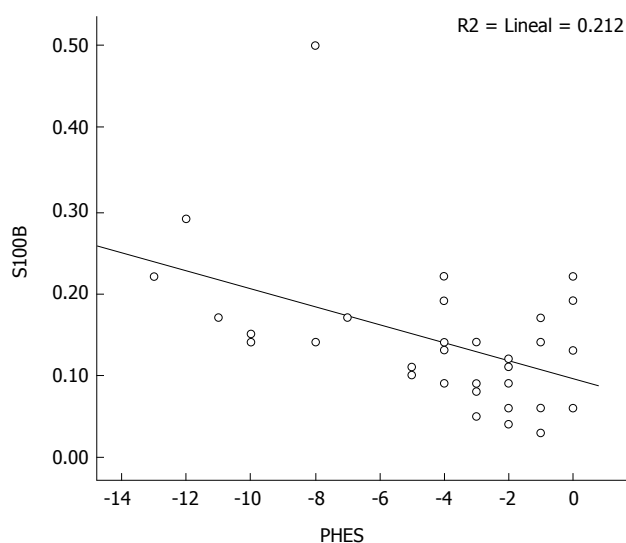
A total of 61 subjects were included, 46 cirrhotic patients and 15 healthy volunteers. The main characteristics of the population are shown in Table 1. Median age was higher in cirrhotics compared to the healthy subjects, most patients were males. The main etiology of cirrhosis was HCV and there was a stepwise increase in Child-Pugh and MELD score according to the presence and severity of HE.

The distribution of S100 $\beta$  serum values according from each group is shown in Figure 1. S100 $\beta$  values were different among all groups, and differences remained significant between groups 1 and 2 ( $P < 0.001$ ), and also between groups 2 and 3 ( $P = 0.016$ ),

**Table 2** Psychometric evaluation

	Healthy subjects	C + non-HE	C + CHE	C + HE	P value
CFF	45.0 (43.0-48.6)	39.9 (39.2-41.4)	36 (33-39)	36 (35-40)	0.000
PHES	0 (-2 to 1)	-2 (-3 to -1)	-9 (-13 to -5)	-9.5 (-12 to -3)	0.000
Digit symbol	33.8 $\pm$ 18.3	28.91 $\pm$ 9.06	18.9 $\pm$ 8.8	13.4 $\pm$ 7.8	0.000
Number connection A	43.6 $\pm$ 21.1	49.5 $\pm$ 19.4	102.8 $\pm$ 66.5	148.8 $\pm$ 85.7	0.000
Number connection B	112.9 $\pm$ 39.1	147.2 $\pm$ 82.8	318.5 $\pm$ 153.03	435.7 $\pm$ 209.9	0.000
Serial dotting	62.3 $\pm$ 22.6	77.43 $\pm$ 18.1	127 $\pm$ 46.9	147.6 $\pm$ 64.3	0.000
Line tracing	95.2 $\pm$ 27.45	95.6 $\pm$ 28.6	193.8 $\pm$ 93.5	167.5 $\pm$ 37.8	0.000

Data presented as absolute frequencies, median (IQR) and mean  $\pm$  SD. C: Cirrhosis; CHE: Covert hepatic encephalopathy; HE: Overt hepatic encephalopathy.



**Figure 2** S100 $\beta$  and PHES correlations between patients with cirrhosis and covert HE and non-covert HE.  $r = -0.413$ ,  $P = 0.019$ . HE: Hepatic encephalopathy; PHES: Psychometric hepatic encephalopathy score.

but not between groups 3 and 4. In cirrhotic patients with HE S100 $\beta$  was higher than in patients without HE [0.18 (0.14-0.28) ng/mL vs 0.11 (0.06-0.14) ng/mL,  $P < 0.001$ ].

The results of the HE psychometric evaluation can be observed in Table 2. There was a clear deterioration in the performance of the CFF and PHES tests in the group with cirrhosis and cirrhosis with CHE or HE, as well as worsening in the individual PHES tests with a statistically significant difference.

In the total population, S100 $\beta$  was found to have a moderate correlation both with PHES ( $\rho = -0.624$ ,  $P < 0.001$ ) and CFF ( $\rho = -0.516$ ,  $P < 0.001$ ). In the subgroup of patients with cirrhosis, S100 $\beta$  showed moderate correlations with PHES ( $\rho = -0.528$ ,  $P < 0.001$ ), as well as with CTP score ( $\rho = -0.515$ ,  $P < 0.001$ ), and MELD score ( $\rho = -0.440$ ,  $P < 0.001$ ), but not with CFF ( $\rho = 0.240$ ,  $P = 0.108$ ) (Figure 2).

ROC curve analysis yielded  $> 0.13$  ng/mL as the best cutoff value of S100 $\beta$  for the diagnosis of CHE and HE, with sensitivity of 83.3% and specificity of 63.6%. Area under the ROC curve (AUROC) was 0.801 ( $P < 0.0001$ ).

## DISCUSSION

HE and CHE are associated with decreased quality of life, impaired working performance, and patients become unfit to safely drive motor vehicles<sup>[16-21]</sup>. Cirrhotic patients with CHE are prone to develop episodes of overt HE when compared to those without CHE (incidence of 56% for those with CHE vs 8% for those without CHE at 3 years of follow up)<sup>[22]</sup>. Moreover, CHE is associated with a decreased survival, and thus it might be considered within the spectrum of complications defining advanced liver failure<sup>[23]</sup>. It is not known whether treatment of CHE may prevent overt HE or improve survival, but it is tempting to hypothesize that early identification of CHE followed by proper treatment should translate into improved quality of life, and have a favorable impact in prognosis. Psychometric tests are the preferred tool to diagnose CHE, and, since the final PHES score is adjusted to age and years of education, the differences of these two variables in our population did not have an impact in the results of our study.

S100 $\beta$  is not entirely specific for the CNS, but it is present in the brain at much higher concentrations than other tissues (80%-90% of the total pool is found within the brain), and as such, this protein can be used as a marker of brain damage<sup>[9]</sup>. Other places where S100 $\beta$  synthesis has been identified are adipose tissue<sup>[24]</sup>, skin and melanoma tumors<sup>[25,26]</sup> and T-lymphocytes<sup>[27]</sup>. S100 $\beta$  protein is secreted by activated astrocytes and the mechanism of secretion remains unknown but astrocyte expression has been reported to be stimulated by interleukin-1 (IL-1) and by cyclic-AMP. The effects of secreted glial S100 $\beta$  depends on its concentration, being neurotrophic at low levels (nanomolar) and neurotoxic at high levels (micromolar)<sup>[9]</sup>. Nanomolar levels of S100 $\beta$  exert a stimulatory effect on astrocytes, causing glial proliferation *in vitro*<sup>[28]</sup>. S100 $\beta$  also accumulates in the extracellular space after astrocyte death or after cellular disintegration of the damaged parenchyma. Under these conditions, the S100 $\beta$  concentration may well be in the micromolar range and the protein may become toxic. This has originated the hypothesis that extracellular accumulation of S100 $\beta$  in ongoing brain



insults can, in combination with other unknown factors, cause a shift in its neuronal attributes from protective to hazardous<sup>[9]</sup>.

We found significant differences in the serum concentrations of S100 $\beta$  between healthy volunteers and patients with cirrhosis. Moreover, in patients with cirrhosis, the levels of S100 $\beta$  increase in the presence of CHE and overt HE. The correlation between S100 $\beta$  and PHES indicates that at lower PHES scores, indicating a higher degree of HE, there is an increased concentration of S100 $\beta$  in serum. This suggests increased permeability of the BBB as the cognitive deterioration of HE progresses, what would determine not only leaking of S100 $\beta$  into the blood circulation, but increased ammonia delivery to the brain, leading to astrocyte activation and swelling. All of these changes (astrocyte activation and swelling, increased BBB permeability) seem to be present in patients with cirrhosis, and, moreover, seem to be more notorious in the presence of CHE and HE. However, our results would suggest that even in the absence of overt HE and CHE, the cirrhotic patient is at a state of astrocyte activation and swelling, and has increased BBB permeability. Brain adaptation to ammonia insults during early stages of cirrhosis and portal hypertension might constitute the counteracting condition preventing CHE or overt HE<sup>[29-31]</sup>. Our understanding of the cellular mechanisms leading to astrocyte dysfunction and neurotoxicity is limited, and it is unclear whether BBB disruption is crucial in the etiopathogenesis of HE, or an inevitable consequence of the disease itself. More studies are needed to better understand the role of S100 $\beta$  in the origin and perpetuation of HE in cirrhotic patients.

When we compared the cirrhotic patients without HE and CHE we found that S100 $\beta$  could differentiate both groups of patients with a fair accuracy, as shown by an AUROC curve of 0.801. Moreover, S100 $\beta$  correlated with the neuropsychometric tests (PHES and CFF), and with the hepatic functional status (MELD and CTP), attesting for its possible role in the evaluation of HE. Our results suggest that S100 $\beta$  can be useful in the diagnosis of HE, precisely in clinical scenarios where the diagnosis of HE remains unclear because behavior changes being subtle, evaluation of highly-educated patients, or when other psychiatric abnormalities (*i.e.*, depression) coexist or cannot be ruled out.

In conclusion, serum concentrations of S100 $\beta$  are higher in patients with cirrhosis when compared to healthy volunteers, and are further increased in the presence of CHE and overt HE. The results suggest the presence of astrocyte dysfunction, and maybe BBB disruption too, in patients with cirrhosis that seems to be more severe and progressive in the presence of HE. S100 $\beta$  showed significant differences between patients with cirrhosis but no encephalopathy at all, and the subgroup presenting with CHE, suggesting its usefulness to identify CHE. Although the AUROC

curve was modest, S100 $\beta$  would potentially be useful in the diagnosis of low-grade HE whenever neuropsychometric tests are suboptimal, or when competing psychiatric differential diagnoses are in place.

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## COMMENTS

### Background

Hepatic encephalopathy (HE) is a frequent complication of cirrhosis, present in 30%-80% of patients. Covert HE has a profound impact in the quality of life of these patients, especially in daily functioning and driving ability, and it affects the sense of well-being. Thus, covert HE is very relevant to patients who suffer from it.

### Research frontiers

The diagnosis and characterization of HE, especially in its low-grade forms, is difficult and requires specialized tests. Serum levels of ammonia were initially considered as a suitable marker of HE; however, they do not correlate with the degree of HE. Therefore, different serum markers for the diagnosis of HE, and particularly covert HE, are urgently needed.

### Innovations and breakthroughs

Little attention has been paid to the use of biochemical markers as a means to detect early stages of HE. The data presented in the current study, indicate that S100 $\beta$  - a molecule released after astrocyte damage - might be a potential marker for covert HE in patients with cirrhosis. Interestingly, the association between high levels of S100 $\beta$  and presence of HE, as well as with the psychometric hepatic encephalopathy score, points towards a novel finding in the field.

### Applications

The determination of S100 $\beta$  levels in serum, together with the standard clinical and psychometric evaluations, might help clinicians to establish the diagnosis of covert HE and therefore a timely treatment for these patients.

### Terminology

S100 $\beta$  is a protein that is primarily synthesized in the brain by the processes of the astrocytes. Although S100 $\beta$  is not entirely specific to the central nervous system, it is present in the brain at much higher concentrations than in other tissues (80%-90% of the total pool is found within the brain), and as such, this protein could be used as an early marker of damage.

### Peer-review

This manuscript mainly describes the association between serum concentrations of S100 $\beta$  and the presence of low-grade HE in patients with liver cirrhosis. The results suggest that S100 $\beta$  might help in the correct characterization of early stages of HE. The content is interesting and meaningful.

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