ORIGINAL ARTICLE

Association between serum albumin and cognitive dysfunction in hepatic encephalopathy: An exploratory data analysis

Kosuke Kaji,* Kiwamu Okita,[†] Kazuyuki Suzuki,[‡] Ikuya Sato,[§] Masaki Fujisawa[§] and Hitoshi Yoshiji*

*Department of Gastroenterology, Nara Medical University, Kashihara, [†]Shunan Memorial Hospital/Yamaguchi University, Yamaguchi, [‡]Division of Hepatology, Department of Internal Medicine, Iwate Medical University, Iwate and [§]Clinical Development Department, ASKA Pharmaceutical Co., Ltd, Tokyo, Japan

Abstract

Background and Aim: Because covert hepatic encephalopathy (CHE) has been shown to affect the prognosis of cirrhotic patients, early diagnosis of hepatic encephalopathy (HE) is a prerequisite for the preservation of patients' quality of life and for prophylaxis of overt HE. The aim of this study was to identify a clinical parameter to predict impairment of cognitive function in cirrhotic patients with early-stage HE.

Methods: We investigated the data from 172 patients with cirrhotic or idiopathic portosystemic shunt (PSS) in phase II/III trials of rifaximin in Japan. Classification and regression trees (CARTs) were constructed to identify clinical profiles related to cognitive dysfunction as indicated by the prolongation of time required for the Number Connection Test (NCT-B).

Results: CART analysis detected age 65 years as the variable for the initial split, and serum albumin level was selected as the variable for the second split among patients aged ≤65 years. In 27 cirrhotic patients aged ≤65 years without PSS, receiver operating characteristic curve analysis revealed that the optimal albumin level cutoff point was 3.05 g/dL, and the area under the curve was 0.80 for the prolongation of NCT-B time, which was higher than that of the branched-chain amino acids-to-tyrosine ratio (0.46), the prothrombin time–international normalized ratio (PT-INR) (0.68), serum ammonia (0.61), and total bilirubin (0.69).

Conclusions: Lower serum albumin level as a clinical biomarker associated with impaired cognitive function may be available as a screening examination for early-stage HE in cirrhotic patients aged ≤65 years without PSS before undergoing neuropsychological tests.

Introduction

Hepatic encephalopathy (HE) is a significant complication of severe acute or chronic hepatic insufficiency, which is characterized by a wide range of changes in mental state from minimal signs of altered brain function to deep coma.[1,2] HE is often caused by hyperammonemia in conjunction with distinct pathophysiological mechanisms, including inflammation, oxidative stress, increased blood–brain barrier permeability, and energy metabolism, as well as putative neurotoxins including short-chain fatty acids, mercaptans, false neurotransmitters (e.g., tyramine, octopamine, and beta-phenylethanolamines), manganese, and γ-aminobutyric acid (GABA).[1–3]

Currently, covert HE (CHE), the earlier stage of HE, is attracting attention. CHE impairs performance on psychometric tests, such as working memory, psychomotor speed, and visuospatial ability, as well as electrophysiological and other functional brain measurements, without any apparent clinical manifestations.[4,5] These cognitive dysfunction and subclinical

symptoms frequently precede further symptoms of HE and cause inconvenience to the patient's daily life. For example, patients with CHE often have sleep disorders and deficits in specific activities, such as driving, which are a danger to themselves and others.[4–6] Therefore, early diagnosis and treatment of CHE is a prerequisite for the preservation of the quality of life and for prophylaxis of overt HE in cirrhotic patients.

A full overview of the different diagnostic modalities of CHE has recently been published. According to the literature, tests used for diagnosing CHE include the Number Connection Tests (NCT) A and B; the Digit Symbol Test; the Block Design Test; the Portosystemic Encephalopathy (PSE) Syndrome Test, providing the Psychometric Hepatic Encephalopathy Score; the Inhibitory Control Test; the Stroop Test; the Scan Test; the Continuous Reaction Time Test; the Cognitive Drug Research test battery; and the Repeatable Battery for the Assessment of Neuropsychological Status.[7–12] Although these tests alone are

noninvasive and feasible for patients in clinical practice, it would be inefficient to apply them to all cirrhotic patients. Moreover, these tests are incapable of discrimination between cirrhosis-based cognitive dysfunction and senility or dementia. Thus, some biomarkers correlated with the above diagnostic modalities are available for screening examination.

To identify a candidate as a biomarker, this study used exploratory data analysis (EDA), a statistical assessment to extract meaningful hypotheses or productive knowledge by using a graphical approach.[13,14] Among various tools for EDA, the classification and regression tree (CART) is available as a statistical method that uses a constructed model to repeatedly recursively divide the explanatory variable involved in the response.[15,16] The factorial structure of the underlying data is visually represented by a "tree." Although EDA is useful for the identification of biomarkers, it has never been used to investigate associations between serum parameters and neuropsychological functions in patients with HE.

The aim of this study was to identify a clinical parameter to predict the prolongation of time required for the NCT (NCT-B) in patients with early-stage HE.

Methods

Study design and ethical approval. This study was undertaken as a stratified analysis of a phase II/III clinical trial of rifaximin to evaluate the relationship between cirrhosis-related clinical parameters and neuropsychological function and identify an available biomarker to predict the presence of early-stage HE.[17] The study protocols conformed to the principles outlined in the 1964 Declaration of Helsinki and its later amendments and were approved by each institution's review board.

Subjects. The subjects of this study were 172 patients from the above clinical trials with cirrhotic or idiopathic portosystemic shunt (PSS). They were diagnosed with early-stage HE (grade I or II HE) according to the criteria of the Inuyama Symposium in Japan and the presence of hyperammonemia (serum ammonia level ≥ 80 μg/dL).[18] Recruitment was limited to patients who were between 20 and 74 years of age at the time of informed consent. The major exclusion criteria were psychiatric comorbidities; any comorbidity or medical history found by the investigator to affect evaluation; acute hepatitis; acute liver failure; acute exacerbation of chronic hepatitis; and laboratory data showing total bilirubin ≥5.0 mg/dL, hemoglobin ≤8 g/dL, serum potassium ≤2.5 mEq/L, or both blood urea nitrogen ≥25 mg/dL and serum creatinine ≥2.0 mg/dL.

Data collection. The following variables were recorded: age; gender; body weight; white blood cell count; platelet count; prothrombin time–international normalized ratio (PT-INR); serum levels of aspartate aminotransferase (AST), alanine aminotransferase, alkaline phosphatase, γ-glutamyl transpeptidase, total protein, albumin, total bilirubin, and creatinine; and branched-chain amino acids (BCAA)-to-tyrosine ratio (BTR). The presence of a portal systemic shunt, esophageal varix, and ascites was evaluated by abdominal images, including ultrasonography, computed tomography, magnetic resonance imaging, and endoscopy. The PSE index was calculated using the following formula, as

previously described: PSE index = (3 × HE coma score + blood ammonia score + asterixis score + NCT-A score)/24.[17] The albumin–bilirubin (ALBI) score was calculated as previously described: ALBI score = (log10 bilirubin [μmol/L] × 0.66) + (Albumin [g/L] × −0.085).[19]

Neuropsychological functions. Neuropsychological functions were assessed by the NCT-B using neuropsychological test system software. The software was distributed by the Japan Society of Hepatology.[20] The hardware consisted of a touch screen tablet, such as an iPad (Apple, Cupertino, CA, USA).

Classification and regression trees. CARTs were constructed to identify the clinical profiles related to the time required for the NCT-B, as previously described.[15,16] The tree plot was visualized with a regression tree model for the NCT-B, and the box plot shows the distribution of observations for nodes and leaves. The preconditions for splitting with CART were as follows: the complexity parameter was set to 0.02, with 40 observations as the minimum number per node.

Receiver operating characteristic curve analysis. Receiver operating characteristic (ROC) curve analysis was performed to validate the predictive performance of the clinical profiles in identifying the impairment of NCT-B time. The amount of prolongation of NCT-B time in the patients differed according to age: age < 45, 51.1 s; 45 ≤ age < 50, 50.2 s; 50 ≤ age < 55, 51.2 s; 55 ≤ age < 60, 62.2 s; 60 ≤ age < 65, 71.3 s; and 65 ≤ age < 70, 87.2 s; and 70 ≤ age < 75, 143.8 s.[20] ROC curve analysis obtained the optimal cutoff point of clinical profiles. Evaluation indices of the ROC curve were calculated for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy on the cutoff point. In addition, the variability of the area under the ROC (AUROC) curves was assessed by using 2000 stratified bootstrap replicates. The summary statistics of AUROC showed the median and two-sided 95% confidence intervals (CIs).

Statistics. The data were expressed as numbers, means ± standard deviation, and medians (range). Differences between groups were estimated using their mean difference and two-sided 95% CIs. Statistical analyses were performed using R software (http://www.Rproject. org/) as previously described.[21] Associations between clinical parameters and neuropsychological function were evaluated by EDA.

Results

CARTs for clinical profiles associated with the time required for the NCT-B. Among the total of 172 patients with early-stage HE, the mean time required for the NCT-B (NCT-B time) was 108.0 ± 48.1 s. Age was selected as the variable for the initial split, and NCT-B time was 87.1 ± 41.0 s in patients aged ≤65 years (Fig. 1a). In 91 patients aged ≤65 years, serum albumin level was selected as the variable for the second split; the NCT-B time was 108.5 ± 46.0 s in patients with serum albumin <2.85 g/dL. In addition, Child–Pugh classification and serum chloride level were selected as the third and fourth splits, respectively.
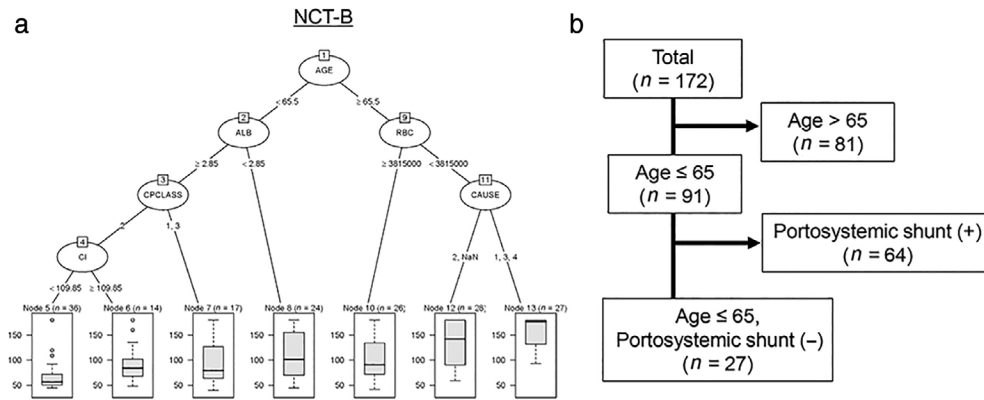
Figure 1 (a) Classification and regression tree for clinical profiles associated with number connection test-B (NCT-B) in 172 Japanese patients with hepatic encephalopathy. (b) The selection of the study population and experimental design. Twenty-seven patients of 81 patients aged >65 years and 64 patients with portosystemic shunt were finally analyzed. ALB, serum albumin level; CPCLASS, Child–Pugh classification; Cl, serum chloride level; RBC, red blood cell count; CAUSE, etiology of liver cirrhosis: 1, hepatitis B; 2, hepatitis C; 3, alcoholic liver injury; 4, the others.

Based on these results, we adopted serum albumin level as a biomarker to predict the impairment of cognitive function, determined as the prolongation of time required for the NCT-B in cirrhotic patients aged ≤65 years.

Characteristic features of patients aged ≤65 years without PSS.
The presence of PSS often causes encephalopathy irrespective of functional hepatic reserve. Thus, to accurately validate the predictive performance for cirrhosis-related cognitive dysfunction, patients with PSS were excluded from the group of 91 cirrhotic patients aged ≤65 years (Fig. 1b). The demographic and clinical characteristics of the patients in the final analysis are presented in Table 1. Twenty-seven cirrhotic patients (16 men and 11 women; mean age, 56.7 ± 8.6 years) were included in the study. Among these subjects, 66.7% had an initial onset of HE, 33.3% had a recurrence of HE, 74.1% developed HE grade I, 25.9% developed HE grade II, and the mean PSE index was 0.34. The majority of patients (70.4%) was classified as Child–Pugh B. The mean NCT-B was 92.2 ± 45.2 s, which was lower than that in cirrhotic patients aged >66 years (133.1 ± 43.1 s), in agreement with the results of CART analysis (Fig. S1,

Supporting information). The mean blood ammonia concentration was 142.2 μg/dL, and the mean BTR was 3.32.

Predictive performance of serum albumin level to identify cognitive dysfunction in cirrhotic patients.
To validate the predictive performance of serum albumin level in identifying the impairment of NCT-B time, we performed ROC curve analysis. In cirrhotic patients aged ≤65 years without PSS, ROC curve analysis revealed that the optimal albumin level cutoff point was 3.05 g/dL (sensitivity, 73.3%; specificity, 91.7%; PPV, 91.7%; NPV, 73.3%; accuracy, 81.5%), and the area under the curve (AUC) value was 0.80 (95% CI, 0.60–0.98) (Fig. 2) for prolongation of NCT-B time. Among all cirrhotic patients, the AUC value to predict prolongation of NCT-B time was 0.63 (95% CI, 0.54–0.71) (cutoff point, 3.15 g/dL; sensitivity, 61.2%; specificity, 62.5%), which was remarkably lower than that in patients aged ≤65 years without PSS (Fig. 2). These findings suggest that serum albumin level effectively identifies cognitive dysfunction in cirrhotic patients aged ≤65 years without PSS.

Efficiency of serum albumin level compared with other parameters related to cirrhosis-based HE.
Next, to evaluate the potential of other parameters related to the occurrence of cirrhosis-based HE to predict cognitive dysfunction, we performed similar analyses of BTR, serum ammonia level, PT-INR, and total bilirubin level. For detection of the prolongation of NCT-B time in cirrhotic patients aged ≤65 years without PSS, the cutoff point was 3.295 for BTR (sensitivity, 46.7%; specificity, 66.7%; PPV, 63.6%; NPV, 50.0%; accuracy, 55.6%), 121.8 μg/dL for serum ammonia level (sensitivity, 73.3%; specificity, 58.3%; PPV, 71.4%; NPV, 61.5%; accuracy, 66.7%), 1.215 for PT-INR (sensitivity, 66.7%; specificity, 66.7%; PPV, 71.4%; NPV, 61.5%; accuracy, 66.7%), and 0.850 for total bilirubin (sensitivity, 93.3%; specificity, 41.7%; PPV, 73.3%; NPV, 66.7%; accuracy, 70.4%) (Fig. S2). The AUC value of serum albumin level (0.80) was higher than the AUC values of BTR (0.46; 95% CI, 0.23–0.69), serum ammonia level

Table 1 Characteristics of patients

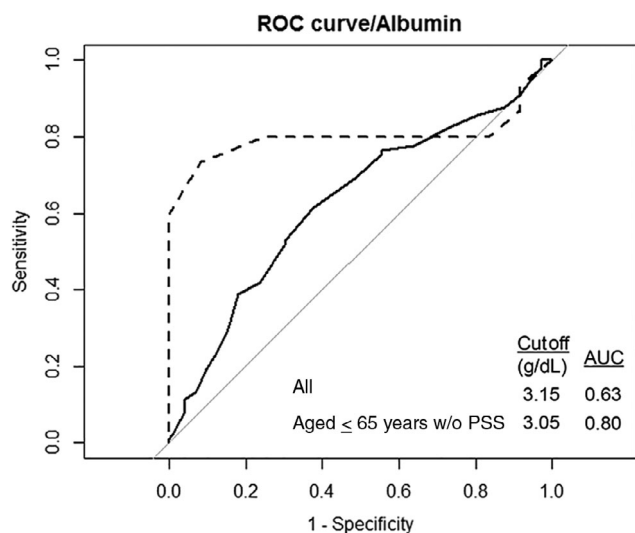| Characteristics	
N	27
Age (years old)	56.7 (8.6)
Gender (male/female)	16 (59.3%)/11 (40.7%)
Body weight (kg)	64.6 (11.6)
Onset of HE (new-onset/recurrent)	18 (66.7%)/9 (33.3%)
PSE index	0.34 (0.12)
HE grade I/II	20 (74.1%)/7 (25.9%)
Child–Pugh classification (A/B/C)	3 (11.1%)/19 (70.4%)/5 (18.5%)
Blood ammonia concentration (μg/dL)	142.2 (41.1)

Figure 2 Receiver operating characteristic (ROC) curve analysis and area under curve (AUC) value for predictive performance of serum albumin level for impairment of number connection test-B. ——, All; ------, aged ≤65 years without portosystemic shunt (w/o PSS).

Figure 4 Receiver operating characteristic (ROC) curve analysis and area under curve (AUC) value for predictive performance of albumin–bilirubin (ALBI) score for impairment of number connection test-B. ——, None; ------, age ≤65 and without portosystemic shunt (w/o PSS).

(0.61; 95% CI, 0.38–0.83), PT-INR (0.68; 95% CI, 0.46–0.87), and total bilirubin (0.69; 95% CI, 0.46–0.89) (Fig. 3), indicating that, compared with other parameters, serum albumin level is distinctly capable of predicting impaired cognitive function in cirrhotic patients aged ≤65 years. In addition, we analyzed the diagnostic performance of ALBI score, which was developed as a simple method to objectively assess hepatic function, for
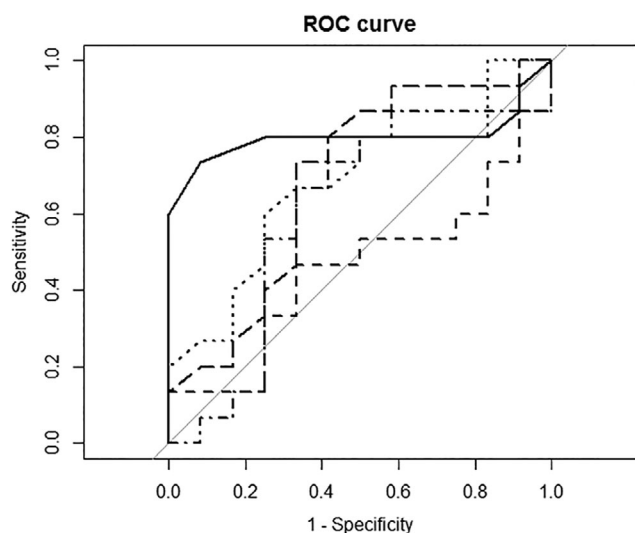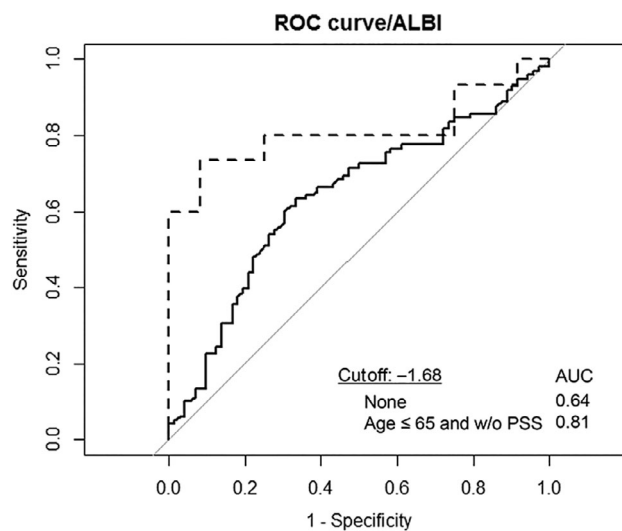
cognitive dysfunction. As shown in Figure 4, in cirrhotic patients aged ≤65 years without PSS, ROC revealed that the optimal ALBI score cutoff point was −1.68 (sensitivity, 66.7%; specificity, 56.3%; PPV, 91.7%; NPV, 73.3%; accuracy, 81.5%), and the AUC value was 0.81 (95% CI, 0.62–0.96) for prolongation of NCT-B time. Among all cirrhotic patients, the AUC value to predict prolongation of NCT-B time was 0.64 (95% CI, 0.55–0.73) (cutoff point, −1.68; sensitivity, 63.3%; specificity, 66.7%). These results indicate that ALBI score is also useful to evaluate cognitive function in cirrhotic patients objectively and easily, and it is not significantly superior to albumin alone.

Discussion

Definitive diagnosis of HE requires advanced clinical skills because potential coexisting disorders must be considered in addition to the underlying liver disease and complications of liver cirrhosis.[1–3,22,23] Previous clinical evidence showed that 54% of patients with cirrhosis and CHE or overt HE had comorbidities that could add to their symptoms in addition to HE, such as electrolyte imbalance, thiamine deficiency, sepsis, and cerebrovascular disorders.[24–29] Patients with overt HE show clinical signs, including cognitive deficits and asterixis, but these are not observed in patients with only HE. Such a backdrop has resulted in the development of various neuropsychological tests for the diagnosis of HE. These tests are clinically effective for detecting subclinical CHE. However, even in the case of CHE, individual differences in intelligence and cognitive abilities influence the results of these tests. Patients with greater intellectual abilities or cognitive reserves may achieve test results within the normal range even when they are experiencing increasing metabolic disequilibrium. On the other hand, these tests may be less accurate for diagnosing CHE in patients with lower cognitive ability. Variation in test results according to individual intelligence requires

Figure 3 Comparison among cirrhosis-related parameters in predictive performance for impairment of number connection test-B in cirrhotic patients aged ≤65 years without portosystemic shunt. Marker: ——, albumin—0.80; ------, branched-chain amino acids-to-tyrosine ratio—0.46; ··········, prothrombin time–international normalized ratio—0.68; ----, ammonia—0.61; —·—, total bilirubin level—0.69. ROC, receiver operating characteristic.

screening examination for parameters relevant to cirrhosis to select the patients who display cognitive dysfunction ascribed to liver cirrhosis.

Our CART analysis demonstrated that a lower level of serum albumin was the major clinical parameter related to liver cirrhosis that was associated with prolongation of the time required for the NCT-B in cirrhotic patients aged ≤65 years. Remarkably, as confirmed by AUC, the predictive value of serum albumin level for detecting prolongation of the time required for the NCT-B was 80.0%, which was higher than that of other cirrhosis-related parameters, including BTR, serum ammonia level, PT-INR, and serum bilirubin level. These results indicate that a lower albumin level may predict cognitive dysfunction in cirrhotic patients aged ≤65 years without PSS. Human serum albumin (HSA) plays a pivotal role in the regulation of oncotic pressure and modulation of inflammatory pathways, as well as microvascular integrity.[30] Clinically, intravenous albumin supplementation has been utilized for the treatment of patients with cirrhosis and ascites.[31–33] The rationale for the use of albumin is based on the recovery of effective circulating volume. Moreover, albumin binds many substances, such as nitric oxide (NO) and reactive oxygen species (ROS), that may be involved in the pathogenesis of both peripheral arterial vasodilatation and cardiac dysfunction in patients with cirrhosis and ascites.[30,34,35] Infusion of albumin can also be beneficial in patients with HE owing to its antioxidant properties and scavenging of ROS. Clinical studies have investigated the efficacy of administration of albumin in patients with HE. Jalan et al. showed that treatment with albumin produced a significant and sustained improvement of HE grade with declining plasma levels of malondialdehyde, a marker of ROS, in patients with alcoholic cirrhosis and diuretic-induced HE compared with treatment with colloids.[36] Furthermore, Setoyama et al. demonstrated that BCAA supplementation could enhance the antioxidative potential by altering the redox state of HSA in cirrhotic patients.[37]

In addition to its antioxidant properties, an outstanding feature of HSA is its physiological ability to bind proinflammatory substances and inflammatory mediators.[30,35] Lipopolysaccharide (LPS) is a surface component of Gram-negative and Gram-positive bacteria that activates the innate immune system via toll-like receptor 4, and LPS-mediated cell activation requires a sequential interaction with several host proteins, including the LPS-binding protein CD14 and the coreceptor MD-2. In the pathogenesis of HE, microbe-derived LPS plays a potentially cardinal role in the development of systemic inflammation and neuroinflammation.[38] In patients with advanced cirrhosis, LPS increases the permeability of the blood–brain barrier and causes astrocyte swelling via production of NO and prostanoids in the brain microglia.[38,39] Clinical evidence suggests that endotoxemia is correlated with the severity of HE and the increased incidence of overt HE.[38,40] Noteworthy evidence is that HSA binds LPS by electrostatic and hydrophobic forces. Our previous study showed that the inactivation of plasma endotoxin was positively correlated with the endotoxin-binding capacity of plasma albumin in patients with Child–Pugh A and B cirrhosis.[41] An experiment with chronically ethanol-fed rats suggested that the increased endotoxin-binding capacity of HSA served as a protective mechanism against the endotoxin.[42] In consideration of this evidence, the LPS–HSA complex could also play a role in the regulation of HE via the inflammatory response to bacterial infection in the cirrhotic state.

In summary, our results show that cirrhotic patients aged ≤65 years with serum albumin levels <3.05 g/dL are at high risk of HE-associated cognitive dysfunction and have to undergo neuropsychological tests. However, this study has several limitations that deserve consideration. First, our data are based on a small number of patients. Further studies are essential to evaluate the diagnostic potential of serum albumin for cognitive function in a larger population. Second, lower albumin level had predictive potential for impaired cognition in cirrhotic patients aged ≤65 years but was not effective in older patients. This finding indicates that using a single parameter of serum albumin level is inadequate to screen for HE-associated cognitive dysfunction in older cirrhotic patients. Finally, this study utilized only the NCT-B, a convenient and common test, as a diagnostic method for CHE. To validate the usefulness of serum albumin level in a screening examination for CHE, the relation of CHE to the results of NCT-B and other neuropsychological tests should be elucidated.

To the best of our knowledge, this is the first study to identify lower serum albumin level as a clinical biomarker associated with impaired cognitive function in cirrhotic patients. We believe that this biomarker is clinically available for a screening examination to determine whether cirrhotic patients aged ≤65 years without PSS should undergo neuropsychological tests for the diagnosis of early-stage HE.

Acknowledgment

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Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website:

Figure S1 The mean time for number connection test (NCT-B) in the groups of patients aged ≤65 years (n = 91) and >66 years (n = 81).

Figure S2 Receiver operating characteristic curve analysis and area under curve (AUC) value for predictive performance of cirrhosis-related parameters for impairment of number connection test-B. w/o PSS, without portosystemic shunt; BTR, branched-chain amino acids-to-tyrosine ratio; PT-INR, prothrombin time–international normalized ratio; Ammonia, serum ammonia level; T-bil; total bilirubin level.