

Lower levels of serum albumin are associated with impairment of cognitive function in cirrhotic patients with early-stage hepatic encephalopathy: An exploratory data analysis of phase II/III clinical trials of rifaximin in Japan

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Abstract

Background Because covert hepatic encephalopathy (CHE) has been shown to affect the prognosis of cirrhotic patients, early diagnosis of HE is a prerequisite for preservation of patients' quality of life and for prophylaxis of overt HE. Currently, neuropsychological tests are used for the diagnosis of early-stage HE including CHE. However, it would be inefficient to apply them to all cirrhotic patients. Thus, some biomarkers correlated with the above diagnostic modalities are available for screening examination. 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 This exploratory data analysis was based on the data from 172 patients with cirrhotic or idiopathic portosystemic shunt (PSS) in phase II/III trials of rifaximin in Japan. Their data at baseline before treatment with rifaximin were utilized to analyze the relationship between cognitive dysfunction and different clinical parameters. We Classification and regression trees (CART) 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 other HE-related parameters including 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 is available as a screening examination for early-stage HE in cirrhotic patients aged ≤ 65 years without PSS before undergoing neuropsychological tests.

Background

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 caused by a combination of distinct pathophysiological mechanisms, including inflammation, oxidative stress, increased blood–brain barrier permeability, and energy metabolism, as well as putative neurotoxins, including ammonia, short-chain fatty acids, mercaptans, false neurotransmitters (e.g., tyramine, octopamine, and beta-phenylethanolamines), manganese, and γ -aminobutyric acid (GABA) - though ammonia [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 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, or the Repeatable Battery for the Assessment of Neuropsychological Status [7–12]. Although these tests 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 employed 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 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 number connection test (NCT-B) in patients with early-stage HE.

Methods

Study design and ethical approval

This study was undertaken as a stratified analysis of phase II/III clinical trials 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 as 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 $\mu\text{g/dL}$) [18]. The subjects were 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; sex; 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 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 = $(3 \times \text{HE coma score} + \text{blood ammonia score} + \text{asterixis score} + \text{number connection test A [NCT-A] score})/24$ [17].

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 [19]. The hardware consisted of a touch screen tablet, such as an iPad (Apple, Cupertino, CA, USA).

Classification and regression trees

CART 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 sec; $45 \leq$ age < 50 , 50.2 sec; $50 \leq$ age < 55 , 51.2 sec; $55 \leq$ age < 60 , 62.2 sec; $60 \leq$ age < 65 , 71.3 sec; and $65 \leq$ age < 70 , 87.2 sec [19]. ROC curve analysis obtained the optimal clinical profiles cutoff point. 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 receiver-operating characteristic (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 \pm 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 [20]. 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 sec. Age was selected as the variable for the initial split, and NCT-B time was 87.1 ± 41.0 sec in patients aged ≤ 65 years (**Figure 1**). 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 sec in patients with serum albumin < 2.85 g/dL. Additionally, Child–Pugh classification and serum chloride level were selected as the third and fourth splits, respectively.

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 irrespectively of functional hepatic reserve. Thus, to accurately validate the predictive performance for cirrhosis-related cognitive dysfunction, patients with PSS were excluded from the 91 cirrhotic patients aged ≤ 65 years. 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%) were classified as Child–Pugh B. The mean NCT-B was 92.2 ± 45.2 sec, which was lower than that in cirrhotic patients aged > 66 years (133.1 ± 43.1 sec), in agreement with the results of CART analysis (**Figure 2**). The mean blood ammonia concentration was 142.2 μ g/dL, and the mean BTR was 3.32.

Table 1. Characteristics of patients

Characteristics	
N	27
Age (years old)	56.7(8.6)
Sex (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 ($\mu\text{g}/\text{dL}$)	142.2(41.1)

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) (**Figure 3**) 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 (**Figure 3**). 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 $\mu\text{g}/\text{dL}$ 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%) (**Supplementary Figure 1**). 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 (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) (**Figure 4**), indicating that compared with other parameters, serum albumin level is distinctly capable of predicting impaired cognitive function in cirrhotic patients aged ≤ 65 years.

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, 21, 22].

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 [23–28]. Patients with overt HE show pathognomonic clinical symptoms, including asterixis, but these are not observed only in patients with 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 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 [29]. Clinically, intravenous albumin supplementation has been utilized for treatment of patients with cirrhosis and ascites [30–32]. 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 [29, 33, 34]. 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. Jaran 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, as compared with treatment with colloids [35]. Furthermore, Setoyama et al. demonstrated that BCAA supplementation could enhance the antioxidative potential by altering the redox state of HSA in the cirrhotic patients [36].

In addition to its antioxidant properties, an outstanding feature of HSA is its physiological ability to bind proinflammatory substances and inflammatory mediators [29, 34]. 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 [37]. 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 [37, 38]. Clinical evidence suggests that endotoxemia is correlated with the severity of HE and the increased incidence of overt HE [37, 39]. 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 [40]. An experiment with chronically ethanol-fed rats suggested that increased endotoxin-binding capacity of HSA served as a protective mechanism against the endotoxin [41]. 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 for HE-associated cognitive dysfunction and have to undergo neuropsychological tests. However, this study has several limitations that deserve consideration. First, 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. Second, 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.

Conclusions

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.

Abbreviations

AST; aspartate aminotransferase, AUC; area under the curve, BCAA; branched-chain amino acid, BTR; branched-chain amino acids-to-tyrosine ratio, CART; classification and regression tree, CHE; covert hepatic encephalopathy, EDA; exploratory data analysis, GABA; γ -aminobutyric acid, HE; hepatic encephalopathy, HSA; human serum albumin, LPS; lipopolysaccharide, NCT; number connection test, NO; nitric oxide, NPV; negative predictive value, PPV; positive predictive value, PSE; portosystemic encephalopathy, PSS; portosystemic shunt, PT-INR; prothrombin time–international normalized ratio, ROC; receiver-operating characteristic, ROS; reactive oxygen species

Declarations

Ethics approval and consent to participate: The study was completed in compliance with Good Clinical Practice guidelines, and the ethical principles were based on the Declaration of Helsinki. The study

protocols were approved by the Institutional Review Boards of the participating institutions, and the study was then carried out after obtaining written, informed consent from each patient according to the phase II/III, multicenter, randomized, evaluator-blinded, active-controlled trial and a phase III, multicenter, open trial [17].

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing Interests: Masaki Fujisawa and Ikuya Sato are employees of ASKA Pharmaceutical Co., Ltd. The other authors have no conflicts of interest.

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Authors' contributions: K.K conducted data analysis and drafted the manuscript. M.F and I.S conducted data analysis. K.O and K.S offered the data support. H.Y designed and supervised the study. All authors read and gave final approval for the manuscript.

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Figures

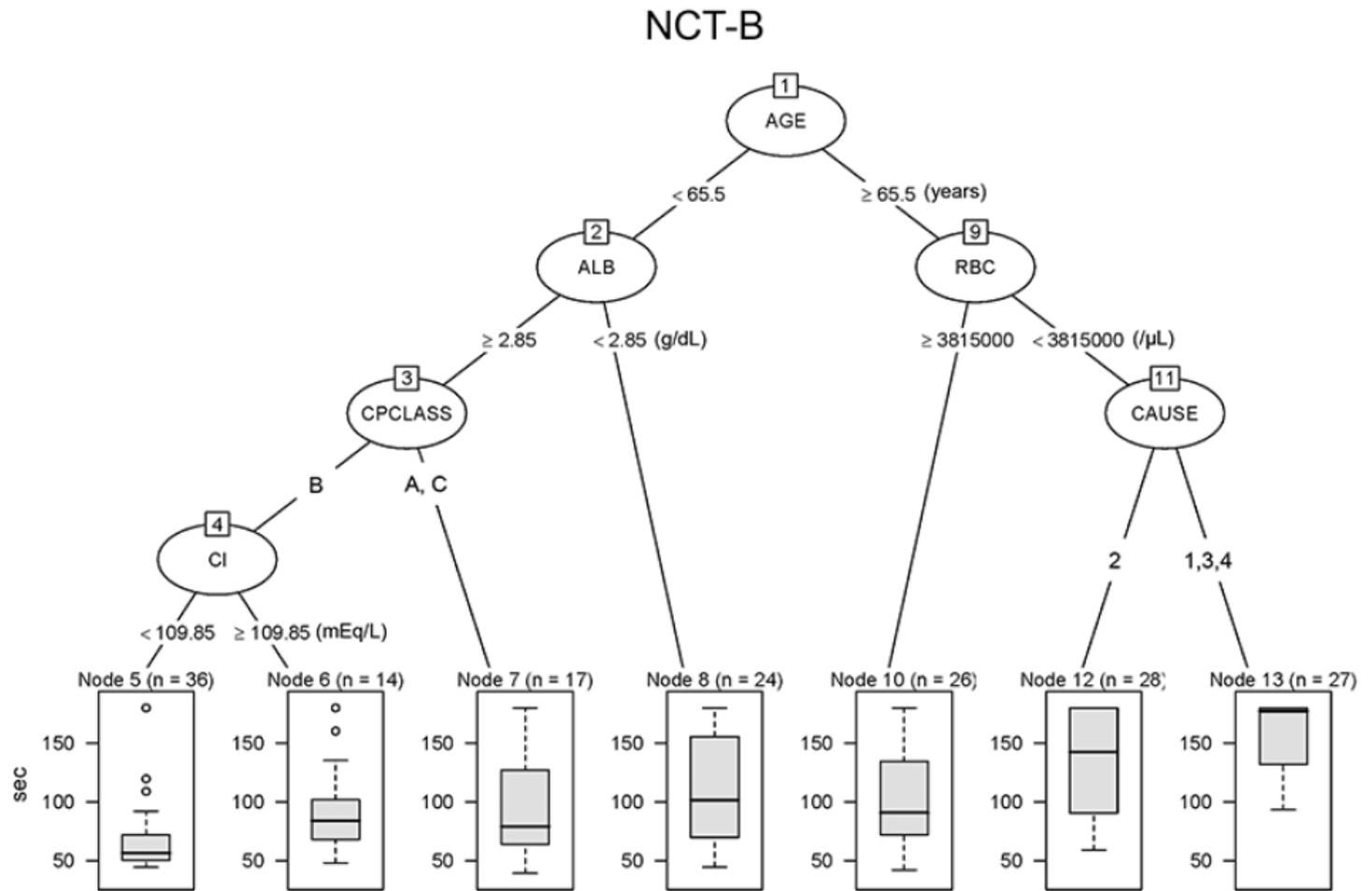


Figure 1

Classification and regression tree for clinical profiles. Clinical parameters were associated with number connection test-B (NCT-B) in 172 Japanese patients with hepatic encephalopathy. 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.

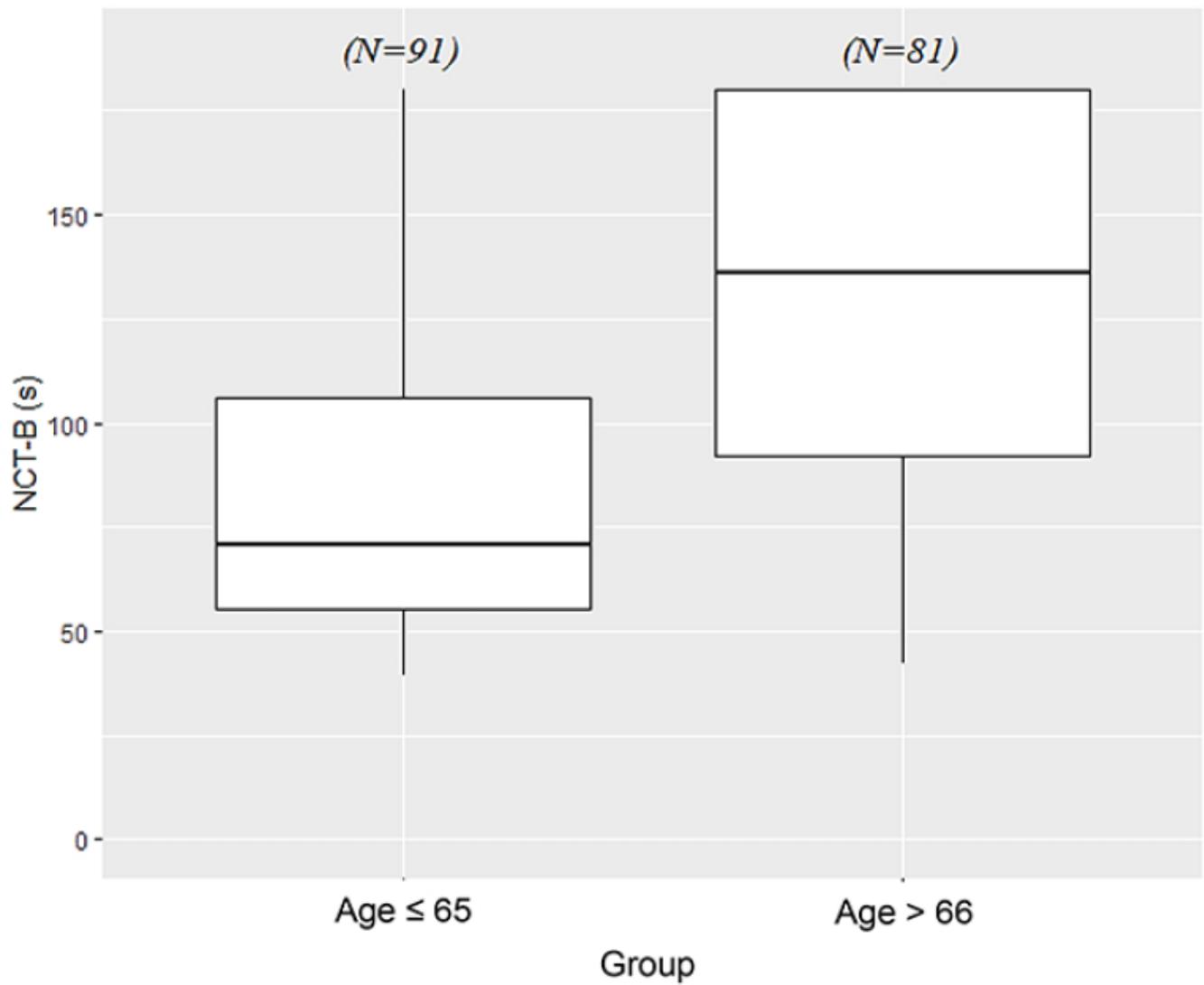


Figure 2

The mean time for number connection test (NCT-B). Comparison the groups of patients between aged \leq 65 years (N=91) and $>$ 66 years (N=81).

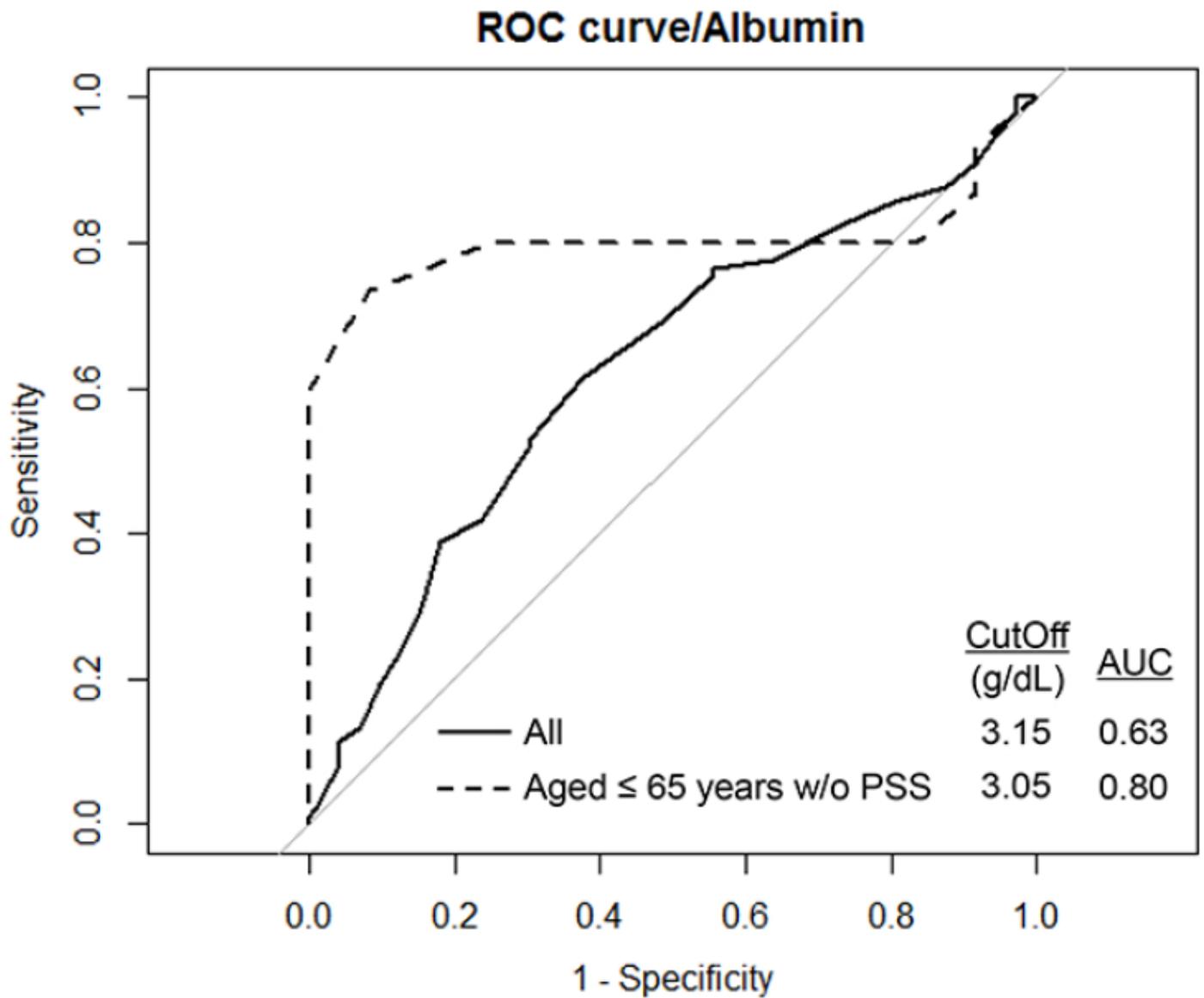


Figure 3

Receiver operating characteristic curve analysis and area under curve (AUC) value. Data analysis for predictive performance of serum albumin level for impairment of number connection test-B. w/o PSS; without portosystemic shunt

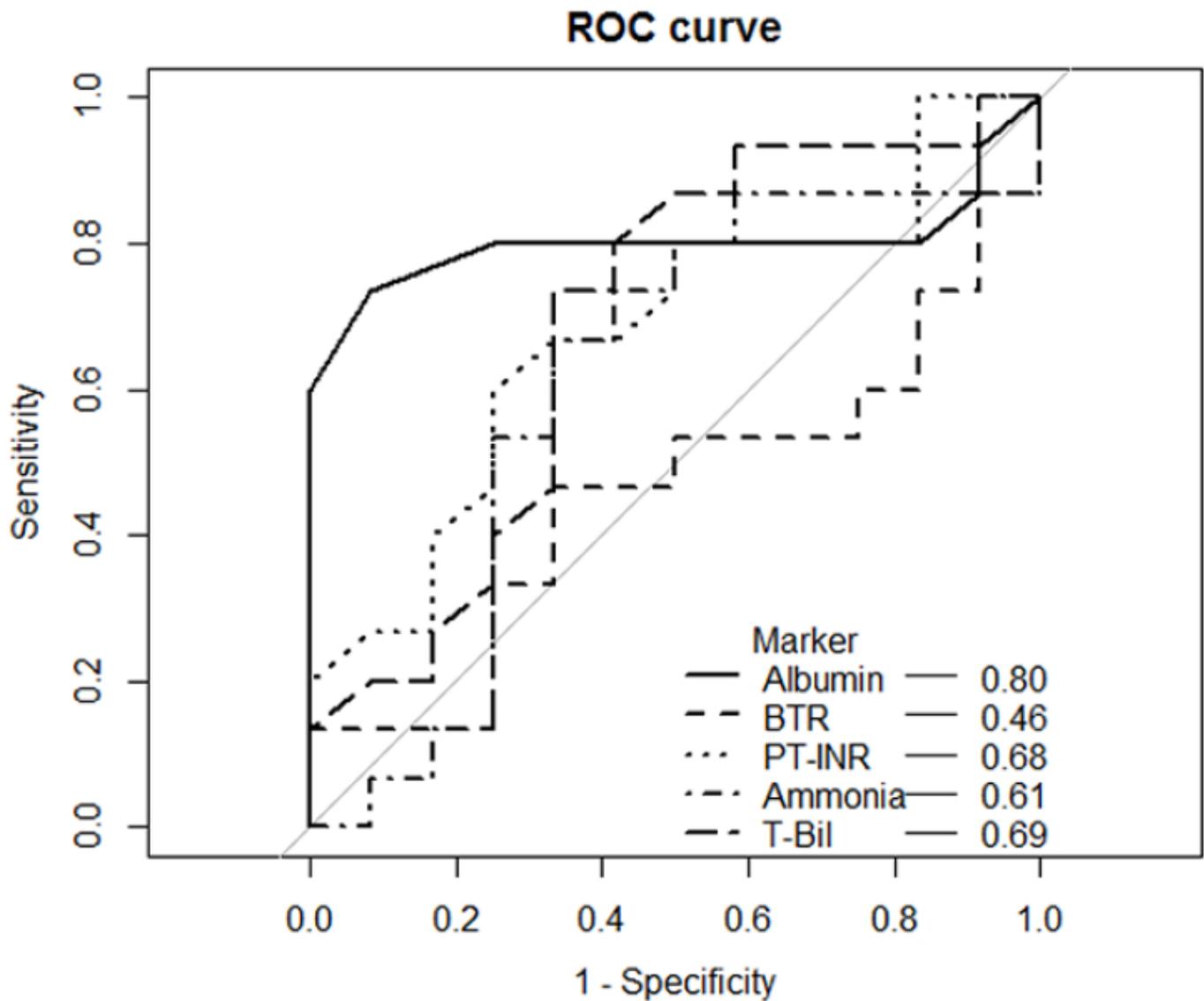


Figure 4

Comparison among cirrhosis-related parameters in predictive performance for impairment of number connection test-B. Data analysis for cirrhotic patients aged ≤ 65 years without portosystemic shunt. Albumin; serum albumin level, BTR; branched-chain amino acids-to-tyrosine ratio, PT-INR; prothrombin time–international normalized ratio, Ammonia; serum ammonia level, T-bil; total bilirubin level.

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