The Molar Ratio of Total Branched-chain Amino Acids to Tyrosine Predicts a Digit Symbol Test Abnormality in Cirrhotic Patients

Yusuke Mizuno[a], Tomohisa Ishikawa[b], Jinya Ishida[b], Akemi Kobayashi[c], Yasuko Konakahara[c], Akiyoshi Kinoshita[a], Hironobu Hama[c], Atsushi Hokari[d], Masayuki Saruta[b]

[a] Department of Gastroenterology and Hepatology, The Jikei University Daisan Hospital, 4-11-1 Izumihon-cho, Komae-shi, Tokyo, 201-8601, Japan

[b] Division of Gastroenterology and Hepatology, Department of Internal Medicine, The Jikei University School of Medicine, 3-19-18 Nishi-shimbashi, Minato-city, Tokyo, 105-8471, Japan

[c] Clinical Nutritional Supports, Jikei University Hospital, 3-19-18 Nishi-shimbashi, Minato-city, Tokyo, 105-8471, Japan

[d] Department of Gastroenterology and Hepatology, The Jikei University Katsushika Medical Center, 6-41-2 Aoto, Katsushika-city, Tokyo, 125-8506, Japan

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E-mail addresses of all co-authors

Tomohisa Ishikawa: ishito@jikei.ac.jp

Jinya Ishida: jikei520208@gmail.com

Akemi Kobayashi: akobayashi@jikei.ac.jp

Yasuko Konakahara: konakahara@jikei.ac.jp

Akiyoshi Kinoshita: aki.kino@jikei.ac.jp

Hironobu Hama: hiro_hama@jikei.ac.jp

Atsushi Hokari: hokari_a@jikei.ac.jp

Masayuki Saruta: m.saruta@jikei.ac.jp

Corresponding Author: Yusuke Mizuno

Department of Gastroenterology and Hepatology, The Jikei University Daisan Hospital, 4-11-1 Izumihon-cho, Komae-shi, Tokyo, 201-8601, Japan

Tel: +81-3-3480-1151

Fax: +81-3-3480-6688

E-mail: ymizuno0319@jikei.ac.jp

Authors' Contributions: Yusuke Mizuno and Tomohisa Ishikawa coordinated the conception and design of this study. Yusuke Mizuno, Jinya Ishida, Akemi Kobayashi and Yasuko Konakahara collected assembly, nutritional, and clinical data. Yusuke Mizuno and Tomohisa Ishikawa conducted the data analysis and drafted the manuscript. Akiyoshi Kinoshita,

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ABSTRACT

Objectives: We aimed to investigate the association between the digit symbol test (DST) and clinical characteristics, including the nutritional status of LC patients. **Methods:** Fifty-nine cirrhotic patients without a history of overt hepatic encephalopathy were retrospectively evaluated. We examined neuropsychological abnormalities (NPAs) using the DST. We also estimated the detailed nutritional status using the Food Frequency Questionnaire (FFQ). The patients were divided into two groups according to their DST status: patients with normal DST scores (DST-Nor group, n=45) and those with abnormal DST scores (DST-Abn group, n=14). The clinical and nutritional findings of the two groups were compared. **Results**: Overall, 14 (23.7%) patients had a DST abnormality. There were significant differences between the two groups in serum albumin (Alb; p=0.0043), valine (Val; p = 0.0016), leucine (Leu; p = 0.0078), isoleucine (Ile; p = 0.0022), the molar ratio of total branched-chain amino acids to tyrosine (BTR; p=0.00025), total-bilirubin (T-Bil; p=0.0071), prothrombin time(%) (PT; p=0.028), and serum sodium (Na; p=0.035). A multivariate analysis found the BTR to be the only independent predictor of a DST abnormality (hazard ratio, 9.24; p<0.031). An FFQ analysis, revealed that the nutritional findings of patients with and without a DST abnormality, were similar. **Conclusion**: The BTR was useful for predicting the risk of NPAs, as defined by a DST abnormality. The risk of NPAs may be estimated by monitoring the BTR.

Key words: Liver cirrhosis, Digit symbol test, BTR, FFQ, hepatic encephalopathy

INTRODUCTION

Various symptoms and clinical findings are indicative of the progression of liver cirrhosis (LC). Among these symptoms, the development of hepatic encephalopathy (HE) is related to a poor prognosis in patients with LC (1-3). Minimal hepatic encephalopathy (MHE), which causes cognitive problems in LC patients, impairs the patient's ability to perform activities of daily living and their quality of life. MHE is an early stage of overt hepatic encephalopathy (OHE) (4-6). The serum ammonia level is commonly measured, and electroencephalography and magnetic resonance spectroscopy are used to evaluate patients with MHE (7).

Some researchers have reported that the results of neuropsychiatric tests (NPTs) are useful for accurately diagnosing MHE (8). The Committee of the Working Party at the Eleventh World Congress of Gastroenterology Vienne (WCOG), the International Society for Hepatic Encephalopathy and Nitrogen Metabolism (ISHEN), and the Japanese Society of Hepatology (JSH) have recommended the use of NPTs for diagnosing MHE (8-10). The WCOG has recommended that at least two of four tests (Number Connection Test-A [NCT-A], Number Connection Test-B [NCT-B], Digit Symbol Test [DST] and Block Design Test [BDT]) be used for the diagnosis of MHE (8, 10). In previous studies, MHE was diagnosed when at least two test results were abnormal (11, 12). In other studies, MHE was diagnosed when the result of at least one of the two tests was abnormal (13, 14). Among the several NPTs available, the DST has been reported to have a high sensitivity and specificity for detecting neuropsychological

problems (14-16). However, the association between the DST and clinical characteristics, including the nutritional status, of LC patients has not been fully clarified.

In the present study, we investigated the association between the DST and clinical characteristics, including the nutritional status of LC patients.

MATERIALS AND METHODS

Study design

Fifty-nine cirrhotic patients without a history of OHE who had been treated at the Division of Gastroenterology and Hepatology, the Jikei University School of Medicine between May 2014 and November 2016 were enrolled in this study. All medical records were retrospectively reviewed for the patients' demographic and clinical data, including the nutritional status. Patients whose condition was complicated with mental disorders, renal dysfunction, bacterial infection, gastrointestinal hemorrhaging, or severe constipation or those treated with an anticonvulsant drug that might affect the cognitive function were excluded from the analysis.

LC was diagnosed based on physical findings, laboratory data, and clinical imaging characteristics. Irregularity and deformity of the shape of the liver was detected via ultrasonography and computed tomography.

The present study was approved by the institutional review board of Jikei University School of Medicine (26-006 7511) and complied with the provisions of the Good Clinical Practice guidelines, the Declaration of Helsinki, and local laws. All enrolled patients provided their written informed consent for participation in the present study.

Physical status and laboratory findings

Demographic and clinical data were extracted or calculated from patient medical records. The

104 body mass index (BMI) and ideal body weight were calculated. According to the assessment
105 criteria for sarcopenia in liver cirrhosis established by the JSH, sarcopenia associated with liver
106 disease is known reduce the muscle mass and muscle strength. The skeletal muscle mass index
107 (SMI), calculated by dividing the left–right sum (cm^2) of the long axis (cm) × short axis (cm)
108 of the iliopsoas muscles at the level of third lumbar vertebra by the height squared (m^2) (the
109 so-called "simple method"), correlated well with the SMI calculated using a muscle mass
110 measurement software program (cut-off value of the SMI calculated by the simple method: 6.0
111 cm^2/m^2 in men, 3.4 cm^2/m^2 in women) (17). While we have no data on the handgrip strength
112 in our LC patients, we evaluated the SMI using a simple method for the evaluation of the
113 muscle mass.

114 Hematological and biochemical tests were performed after fasting for more than 8 h.
115 The hematological analyses included the white and red blood cell counts, and platelet counts;
116 in addition, the hematocrit and hemoglobin levels were determined. The levels of serum alanine
117 transaminase (ALT), serum albumin (Alb), total bilirubin (T-Bil), creatinine, serum ammonia
118 (NH_3), branched-chain amino acids to tyrosine ratio (BTR), prothrombin time (PT%), and C-
119 reactive protein (CRP) were determined. As an amino acids analysis, the levels of valine (Val),
120 leucine (Leu), isoleucine (Ile), methionine (Met), tyrosine (Tyr), phenylalanine (Phe), histidine
121 (His), tryptophan (Trp), ornithine (Orn), lysine (Lys), and arginine (Arg) were also determined.
122

123 *Performance of the DST*

124 The DST was performed using a tablet-type device developed by Otsuka Pharmaceutical
125 (Tokyo, Japan), Kokuyo (Osaka, Japan), and ISB (Tokyo, Japan); the tablet and NPT program
126 were provided by the JSH. The DST values were estimated based on deviations from standard
127 age- and sex-dependent values. Each patient's DST data were compared with the 10^{th} and 90^{th}
128 percentile cut-off values for healthy individuals, with 5-year quartile ranges (5, 18). The DST
129 is performed after several days of alcohol abstinence for patients with alcoholic liver cirrhosis.
130
131 *The evaluation of the nutritional status*
132 We calculated the total daily energy expenditure (TEE) using the Harris-Benedict equation.
133 The activity factor was 1.3, and the injury/stress factor was 1.0, as enrolled patients had chronic
134 liver disease but were ambulatory and were able to visit the hospital outpatient clinic by
135 themselves (19, 20). We estimated the detailed nutritional status using the Food Frequency
136 Questionnaire (FFQ), which was administered at the initiation of nutritional support and
137 performed under the supervision of a nationally registered dietician, according to our study
138 schedule. The FFQ was also used to calculate the nutrient intake, estimated intake energy per
139 day (EIE), and the usual daily energy ratio of nutrients (PFC ratio). The estimated intake
140 amount of dietary fiber, n-3/n-6 unsaturated fatty acids and salt were calculated. The intake
141 amount of each nutrient was estimated using a software program adapted to Japanese eating

and living habits (Excel Eiyou-kun version 6.0; Kenpakusha, Tokyo, Japan). We compared the detailed nutritional data of our patients to the estimated energy requirements (EER), the estimated average requirements (EAR), and adequate intake values (AI), as determined by the Japanese Ministry of Health, Labor and Welfare (21, 22).

Oral branched-chain amino acid (BCAA) supplementation

Patients ingested BCAA granules (LIVACT; EA Pharma Co., Ltd., Tokyo, Japan) that contained 952 mg of L-isoleucine, 1904 mg of L-leucine, and 1144 mg of L-valine per sachet or BCAA powder mix (Aminoleban EN; Otsuka Pharmaceutical, Tokyo, Japan) that contained 2037 mg of L-leucine, 1922.5 mg of L-isoleucine, 1602 mg of L-valine, 242.5 mg of L-lysine, 302 mg of arginine, 187.5 mg of histidine, and 73.5 mg of tryptophan per package. Patients ingested one sachet after each meal or one to three packages daily.

Statistical analyses

We used EZR (23), a graphical user interface for R, version 1.29, to perform the statistical analysis (The R Foundation for Statistical Computing, Vienna, Austria). For individual variables, all data were expressed as the mean and standard deviation (SD), unless stated otherwise. Categorical variables were compared using the chi-squared test, while continuous variables were compared using the Mann–Whitney U test. To assess the factors predicting a

DST abnormality, logistic regression analyses were used to calculate odds ratios (ORs) and 95% confidence intervals (CIs). Only variables with a p-values of <0.05 in a univariate analysis were included in the multivariate analysis.

A receiver operating characteristics (ROC) was also generated to determine the optimal cut-off value of continuous variables. P-value of < 0.05 were considered to indicate statistical significance.

RESULTS

Etiology and clinical findings

The patient demographics and clinical characteristics are shown in Table 1. The mean age of the patients was 66 years old. Thirty-six (61%) patients were men, and 23 (39%) were women. The mean BMI was 24.0 ± 4.7 kg/$m^{2.}$ The most common etiology of LC was hepatitis C (n=24, 40.7%), followed by alcohol-associated hepatitis (n=17, 28.8%), NASH (n=6, 10.1%), and hepatitis B (n=4, 6.8%). Of the 59 patients enrolled, none showed portosystemic shunt on dynamic-enhanced computed tomography scans or ultrasonography. Thirty-one patients (52.5%) had a history of hepatocellular carcinoma (HCC) treatment. Twenty-seven patients (45.8%) used BCAA supplementation. Thirteen patients took 1 BCAAs granule sachet after each meal, whereas BCAA powder mix was used by 14 patients (11 patients took 1 package daily as a late-evening snack, 1 patient took 2 packages per day, and 2 patients took 3 packages per day). The Child–Pugh stages of the patients were as follows: stage A (n=37), B (n=14), and C(n=8). The mean Child–Pugh score (CP-S) was 6.8 ± 2.1. The mean Alb (3.4 ± 0.5 g/dL), BTR (3.9 ± 1.6), and platelet counts (11.5 ± 5.9 × 10^4/μL) were lower than the reference values.

DST results and their correlation with clinical characteristics

We divided patients into two groups based on the reference values (DST-Abn group, n=14, DST-Nor group, n=45). The correlation between the DST status and the clinical characteristics

is shown in Table 2 and Figure 1. The CP-S of the DST-Abn group was significantly higher than that of the DST-Nor group (6.3 ± 1.3 vs. 8.8 ± 2.9, p = 0.0052). The DST-Abn group also had markedly higher T-Bil levels than the DST-Nor group (2.6 ± 1.7 vs. 1.2 ± 0.5, p = 0.0071). In addition, the levels of Alb (3.0 ± 0.5 vs. 3.5 ± 0.5, p = 0.0043), PT (62.4 ± 20.9 vs. 76.7 ± 14.3, p = 0.028), serum sodium (137.6 ± 3.5 vs. 140.0 ± 2.6, p = 0.035) , Val (168.6 ± 60.9 vs. 226.9 ± 49.0, p = 0.0016), Leu (87.1 ± 36.2 vs. 122.3 ± 31.2, p = 0.0078), and Ile (49.5 ± 19.1 vs. 69.0 ± 17.0, p = 0.0022) as well as the BTR values (2.8 ± 1.1 vs. 4.3 ± 1.6, p = 0.00025) were notably lower in the DST-Abn group than in the DST-Nor group. There were no significant differences between the two groups regarding the use or non-use of BCAA supplementation (p = 0.776).

The results of the multivariate analysis are shown in Table 3. While univariate analyses identified significant differences in CP-S, the CP-S is composed of Alb, T-Bil, PT, ascites, and hepatic encephalopathy and was therefore excluded from the multivariate analysis. As a result, a BTR of <2.92 was found to be an independent predictor of a DST abnormality (hazard ratio [HR], 9.24; p < 0.031).

Given the possible effect of BCAA supplementation on the DST and BTR, LC patients not taking BCAA supplementation were divided into two groups according to the DST result. The clinical characteristics of the LC patients without BCAA supplementation who had normal and abnormal DST results are shown in Table 6. There was a significant difference in the BTR

206 between the two groups in a univariate analysis (p = 0.0385). A multivariate analysis was not
207 performed due to the small number of subjects.
208
209 *The estimation of the nutritional status using the FFQ*
210 Overall, 41 enrolled LC cases completed the FFQ. The remaining 18 patients were unable to
211 complete the FFQ because of the amount of time required. We estimated the nutritional status
212 of 41 patients using physical measurements and the FFQ (Table 4). The TEE was not
213 significantly different from the EIE (men: 1769.4 ± 309.3 kcal vs. 1932.7 ± 533.4 kcal, p >
214 0.05; women: 1515.7 ± 149.7 kcal vs. 1636.9 ± 455.8 kcal, p > 0.05). The protein ingestion
215 values (men: 1.2 ± 0.3 g/kg; women: 1.1 ± 0.4 g/kg) were not significantly different from the
216 recommended values. The fat energy ratio (men: 28.6% ± 5.2%; women: 30.2% ± 4.5%) was
217 significantly higher than the AI (20.0%-25.0%). We found that 36.6% of the patients had a fat
218 energy ratio of >30% (n=15/41). The dietary fiber intake of men (16.2 ± 6.0 g/day; vs. women,
219 16.2 ± 4.4 g/day) was significantly less than the AI (>19 g/day, p = 0.03; vs. women, >17 g/day,
220 p = 0.60).
221
222 *The nutritional condition according to the DST status*
223 In the 41 LC patients who completed the FFQ, we compared the nutritional condition between
224 the DST-Abn and DST-Nor groups. There were only 7 patients (2 men and 5 women) in the

DST-Abn group. We therefore judged that it was impossible to consider male and female patients separately. Nutritionally, the DST-Abn and DST-Nor groups were similar (Table 5). Based on the FFQ analysis, both groups had fat energy ratios that were above the AI, and both groups demonstrated insufficient dietary fiber intake.

DISCUSSION

HE serves as a prognostic factor in patients with LC. Although studies have reported that half of MHE patients develop OHE within three years (1, 3), the diagnostic methods and detailed pathogenesis of MHE remain to be clarified. Based on these limitations, as well as the adverse effects on the patient's quality of life, we consider the establishment of diagnostic criteria for MHE to be imperative.

Most LC patients have protein energy malnutrition (PEM) and accelerated resting energy expenditure. Drastic changes in the metabolic nutrient ratio are observed in patients with liver dysfunction, in addition to a decreased carbohydrate ratio and increased fat ratio (24, 25). The absorption of aromatic amino acids into the nerve cells is also stimulated due to an amino acid imbalance, which leads to higher-brain-function restrictions and the development of HE (26-28). Practical nutritional support, which can help overcome nutritional problems, improves the prognosis of LC patients (25, 29, 30).

In the present study, we selected the DST as an indicator of earlier neurophysiological problems in cirrhotic patients. The approach used in this study conformed to the WCOG report, which requires that a neurophysiological assessment be used to diagnose MHE after excluding any other brain disorders (8). The NPTs, which are recommended by the WCOG, ISHEN, and JSH, are a valuable and useful method for diagnosing MHE (8-10). Michitaka et al. reported that abnormalities in 2 NPT items has 80% specificity for MHE, while abnormalities in ≥3

items has 95% specificity (12). Some studies have reported that more than two abnormal test items in the NCT-A/B, DST, and BDT are indicative of MHE (31). Li et al. reported that MHE could be diagnosed with 76.9% sensitivity and 96.3% specificity using the combination of the NCT-A and DST (15). Weissenberg et al. reported that, among the NPTs, the DST has high sensitivity (80.0%) and specificity (96.5%) for the diagnosis of MHE (16). We therefore selected the DST to evaluate early neurophysiological problems.

In our study, 14 (23.7%) patients were classified into the DST-Abn group, indicating that patients in this group are at risk of developing MHE. Although the CP-S in the DST-Abn group was higher than that in the DST-Nor group, four patients in the DST-Abn group had a Child–Pugh classification of A. Researchers are particularly interested in such groups of patients, as these patients have a relatively good clinical liver function. The values of Val, Leu, Ile, and the BTR in the DST-Abn group were significantly lower than in the DST-Nor group. Furthermore, a multivariate analysis demonstrated that a BTR <2.92 was independently associated with DST abnormality. Recently, Hanai et al. reported that the presence of sarcopenia and a low BCAAs level (<327 nmol/ml) was independently associated with MHE in LC patients, supporting our findings (32). Previous studies reported that the serum BCAA values might be remarkably decreased in patients with compensated LC in Child-Pugh class A; in particular, lower valine values were closely associated with the liver prognosis and mortality (33-35). The ammonia detoxification pathway includes the urea cycle in the liver and the

glutamine synthesis pathway in the skeletal muscle and brain. In cirrhotic patients, while the ability to process ammonia by the urea cycle is reduced, the detoxification of ammonia to glutamine in skeletal muscle, brain, and likely the lungs is activated. When glutamate and ammonia react to form glutamine, ammonia is processed. However, an increase in the concentration of glutamine induces hepatic encephalopathy by causing astrocyte swelling and cerebral hypertension in the brain. Furthermore, in skeletal muscle, BCAAs (Val, Leu, Ile) are metabolized to acetyl-CoA and succinyl-CoA and converted to α-ketoglutarate in the Krebs cycle. α-ketoglutarate itself is also a source of glutamate, generating ammonia through the process of producing glutamine. However, the produced glutamine is metabolized in the intestine and kidney to ammonia and glutamate, and glutamate in also decomposed into α-ketoglutarate and ammonia. Even if the ammonia concentration decreases in a certain organ, ammonia can be consequently produced in another organ, resulting in a vicious cycle in which the ammonia level in the body does not decrease (34, 36). We hypothesize that the neuropsychological dysfunction and MHE associated with LC is related to the fractional imbalance of amino acids in these patients. In fact, Akahoshi et al. demonstrated that NPT scores (including those of the NCT, BDT, and DST) were negatively associated with the BTR in patients undergoing liver transplantation and that the BTR in patients with MHE was significantly lower than that in those without MHE (37). We therefore suggest that the BTR is a useful parameter for predicting the development of cognitive problems similar to MHE.

The FFQ have been used in nutritional epidemiological studies to investigate the association between food and chronic diseases (38-44). Evaluating the dietary intake helps physicians and dieticians understand poor food habits, dietary choice, and excessive consumption. Suitable management of malnutrition involves establishing daily a diet that includes a restricted calorie intake and diverse nutrient consumption (45). Using the FFQ, the quantity of daily intakes was calculated based on the portion size and consumption frequency. The TEE/EIE ratios in men and women in the present study were 1.0 ± 0.5 and 1.0 ± 0.3, respectively. The EIE as estimated by the FFQ and the TEE as calculated by the Harris-Benedict equation were almost same value. Furthermore, the EIE/BW and protein intake in this study were similar to the ideal values established in the European Society for Parenteral and Enteral Nutrition (ESPEN) guidelines and in the ISHEN recommendations (29, 30).

The ideal AI for the fat energy ratio is reported to be 20%-25% (21, 22). In the present study, regardless of the presence of an abnormal DST, the fat energy ratio (men: 28.6% ± 5.2%; women: 30.2% ± 4.5%) exceeded that of the ideal AI (21, 22). Consistent with our results, Tajika et al. showed that the rate of fat oxidation in cirrhotic patients (47.8%), as determined by an indirect calorimeter, was higher than that in the control group (26.6%) (24). In addition, the lipid uptake rate of NASH patients estimated using a 4-day diet diary was reported to be 35% of the total energy calories (38). The findings of these reports are similar to our results, indicating the accuracy of the FFQ results in our study.

In the present study, the intake of dietary fiber by the enrolled patients was lower than the AI. Dietary fiber reduces gut digestion, passage time, and ammonia absorption. A previous study reported a close relationship between gut flora and the ingestion of dietary fiber, with a reduced fiber consumption resulting in gut flora changes (46-48). Nutrient ratios and dietary fiber ingestion should be further investigated as a means of preventing cognitive problems, including DST abnormalities and even HE.

There were no marked nutritional differences between the DST-Abn and DST-Nor groups in our study. In contrast, a recent report, by Zhu et al. showed that the total protein intake, total unsaturated fatty acid intake, and total carbohydrate intake were associated with changes in the mean total latency and number of errors in a symbol digit substitution test (49). The small sample size of our study may have affected the statistical analyses.

This study was associated with several limitations. First, the study was retrospective in nature and included a relatively small number of patients who were managed in a single center. Although the multivariate analysis showed the BTR to be an independent marker for detecting a DST abnormality, this method is of limited value in a retrospective, small-scale study. These methodological drawbacks may reduce the strength of our statistical conclusions. Second, we did not directly compare the FFQ and the measurement results, including the oxidation rates of carbohydrate, fat, and protein, respiratory quotient and resting energy expenditure, as determined by an indirect calorimeter. Third, liver cancer may affect the nutritional status.

Nevertheless, LC patients with liver cancer and a good performance status are often encountered in actual clinical practice and participated in the current study. Thus, a large-scale, prospective study is required to confirm our findings.

In conclusion, we found that the pathophysiology of a DST abnormality is closely related to decreases in the BTR level. The BTR is the most useful predictor of a DST abnormality in LC patients. The NPA risk may be estimated by monitoring the BTR.

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Conflicts of Interest and Funding Disclosure

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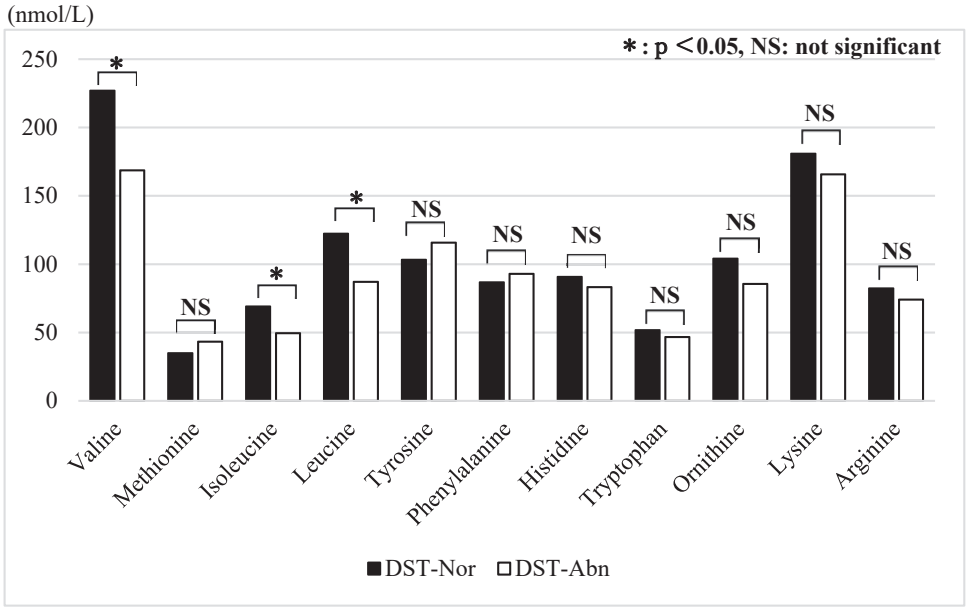
Figure legends

Figure 1. The results of the amino acid analysis according to the DST status. Significant differences between the two groups were noted in valine (p=0.0016), leucine (p=0.0078), and isoleucine (p=0.0022). The tyrosine value was similar between the two groups.

Figure 1.



DST-Nor, normal digit symbol test; DST-Abn, abnormal digit symbol test.

Table 1. The clinical characteristics and laboratory findings of the enrolled patients

Characteristics	All patients (n = 59) Mean ± SD or n (%)
Age (years)	66.1 ± 10.7
Sex (M/F)	36 / 23
BMI (kg/m^2)	24.0 ± 4.7
BMI >25	19 (32.2)
Number of cases with reduced muscle volume	10 (16.9)
Etiology	
HBV	4 (6.8)
HCV	24 (40.7)
Alcohol	17 (34.7)
NASH	6 (10.2)
others	8 (13.6)
Child–Pugh score	6.8 ± 2.1
Child–Pugh classification (A/B/C)	37 / 14 / 8
MELD score	9.3 ± 4.8
Complication	
HCC	31 (52.5)
Ascites	12 (20.3)
Number of cases using BCAA supplementation	27 (45.8)
BCAA granules, three sachets daily	13 (22.0)
BCAA powder mix, one/two/three packages daily	11 (18.6) /1 (1.7) /2 (3.4)
Concomitant medications	
Lactulose	5 (8.5)
Antibiotics	5 (8.5)
Zinc sulfate	3 (5.1)
L-Carnitine	0 (0.0)
Molecular-targeted agents for HCC	0 (0.0)
＜Laboratory findings＞	
ALT (U/L)	39.1 ± 31.2
Albumin (g/dL)	3.4 ± 0.5
Total-bilirubin (mg/dL)	1.5 ± 1.1
Platelet (×10^4/μL)	11.5 ± 5.9
Prothrombin time (%)	73.3 ± 17.0
Na (mmol/L)	139.4 ± 3.0

NH_3（μg/dL）	56.2 ± 27.4
CRP (mg/dL)	0.44 ± 0.64
Amino Acids, standard value	
Valine (nmol/L), 158.4 ~ 287.7	212.6 ± 57.4
Leucine (nmol/L), 80.9 ~ 154.3	113.7 ± 35.5
Isoleucine (nmol/L), 41.3 ~ 84.9	64.2 ± 19.3
Methionine (nmol/L), 19.2 ~ 32.7	37.0 ± 14.3
Tyrosine (nmol/L), 50.2 ~ 82.6	106.3 ± 31.1
Phenylalanine (nmol/L), 45.7 ~ 76.5	88.3 ± 26.6
Histidine (nmol/L), 67.9 ~ 97.1	88.9 ± 15.8
Tryptophan (nmol/L), 41.4 ~ 65.5	50.5 ± 14.6
Ornithine (nmol/L), 43.2 ~ 95.7	99.5 ± 31.2
Lysine (nmol/L), 118.7 ~ 257.0	177.1 ± 44.4
Arginine (nmol/L), 46.0 ~ 121.7	80.3 ± 28.4
BTR	3.9 ± 1.6

SD, standard deviation; BMI, body mass index; HBV, hepatitis B virus; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis; MELD score, model for end-stage liver disease score; HCC, hepatocellular carcinoma; BCAAs, branched-chain amino acids; ALT, alanine transaminase; Na, serum sodium; NH_3, serum ammonia; CRP, C-reactive protein; BTR, branched-chain amino acid to tyrosine ratio.

2

3 Table2. The clinical characteristics of patients with normal and abnormal digit symbol test
4 results

Characteristics	DST-Nor Mean ± SD or n (%)	DST-Abn Mean ± SD or n (%)	p value
No. of cases	45	14	
Age (years)	66.5 ± 11.3	64.6 ± 8.8	p = 0.52
Sex (M/F)	29 / 16	7 / 7	
BMI (kg/m^2)	23.9 ± 4.8	24.5 ± 4.4	p = 0.65
Number of cases with reduced muscle volume	6 (13.3)	4 (28.6)	p = 0.24
Child–Pugh score	6.1 ± 1.3	8.8 ± 2.9	p = 0.0052
Child–Pugh classification (A/B/C)	33 / 10 / 2	4 / 4 / 6	
MELD score	8.6 ± 4.3	11.6 ± 5.7	p = 0.095
Complication			
HCC	25 (55.6)	6 (42.9)	p = 0.542

Ascites	6 (13.3)	6 (42.9)	p = 0.026
Number of cases using BCAA supplementation	20 (44.4)	7 (50.0)	p = 0.776
BCAA granules, three sachets daily	10 (22.2)	3 (21.4)	
BCAA powder mix, one/two/three packages daily	9 (20.0)/0(0)/0(0)	2(14.3)/1(7.1)/2(14.3)	
Concomitant medications			
Lactulose	2 (4.4)	3 (21.4)	p = 0.081
Antibiotics	2 (4.4)	3 (21.4)	p = 0.081
Zinc sulfate	3 (6.7)	0 (0.0)	p = 1.00
<Laboratory findings>			
ALT (U/L)	36.2 ± 25.1	48.3 ± 45.7	p = 0.36
Albumin (g/dL)	3.5 ± 0.5	3.0 ± 0.5	p = 0.0043
Total-bilirubin (mg/dL)	1.2 ± 0.5	2.6 ± 1.7	p = 0.0071
Platelet ($\times 10^4/\mu L$)	11.4 ± 5.5	11.9 ± 7.4	p = 0.80
Prothrombin time (%)	76.7 ± 14.3	62.4 ± 20.9	p = 0.028
Na (mmol/L)	140.0 ± 2.6	137.6 ± 3.5	p = 0.035
NH_3 (μg/dL)	56.8 ± 28.5	54.1 ± 24.7	p = 0.73
CRP (mg/dL)	0.4 ± 0.6	0.7 ± 0.7	p = 0.122
Amino Acids, standard value			
Valine (nmol/L), 158.4 ~ 287.7	226.9 ± 49.0	168.6 ± 60.9	p = 0.0016
Leucine (nmol/L), 80.9 ~ 154.3	122.3 ± 31.2	87.1 ± 36.2	p = 0.0078
Isoleucine (nmol/L), 41.3 ~ 84.9	69.0 ± 17.0	49.5 ± 19.1	p = 0.0022
Methionine (nmol/L), 19.2 ~ 32.7	34.9 ± 10.6	43.4 ± 21.6	p = 0.215
Tyrosine (nmol/L), 50.2 ~ 82.6	103.3 ± 29.5	115.8 ± 35.3	p = 0.284
Phenylalanine (nmol/L), 45.7 ~ 76.5	86.8 ± 22.1	93.0 ± 38.2	p = 0.825
Histidine (nmol/L), 67.9 ~ 97.1	90.7 ± 15.4	83.2 ± 16.3	p = 0.175
Tryptophan (nmol/L), 41.4 ~ 65.5	51.8 ± 13.2	46.8 ± 18.6	p = 0.364
Ornithine (nmol/L), 43.2 ~ 95.7	104.0 ± 32.7	85.6 ± 21.9	p = 0.112
Lysine (nmol/L), 118.7 ~ 257.0	180.8 ± 46.9	165.7 ± 34.8	p = 0.376
Arginine (nmol/L), 46.0 ~ 121.7	82.3 ± 28.6	74.1 ± 28.1	p = 0.339
BTR	4.3 ± 1.6	2.8 ± 1.1	p = 0.00025

DST-Nor, normal digit symbol test; DST-Abn, abnormal digit symbol test; BMI, body mass index; MELD, model for End-stage Liver Disease; HCC, hepatocellular carcinoma; BCAAs, branched-chain amino acids; ALT, alanine transaminase; Na, serum sodium; NH_3, serum ammonia; C-reactive protein; BTR, branched-chain amino acids to tyrosine ratio.

Table 3. The multivariate analysis of factors associated with a DST abnormality

parameter	Odds ratio	95% CI	p value
BTR ＜2.92	9.24	1.23 - 69.2	0.031
Alb ＜2.90	1.63	0.175 - 15.2	0.669
T-Bil ＞1.70	3.10	0.581 - 16.6	0.185
PT ＜70	0.772	0.101 - 5.90	0.803
Na ＜138	3.34	0.519 - 21.5	0.204
The presence of ascites	1.13	0.100 - 13.0	0.923

DST, digit symbol test; 95%CI, 95% confidence interval; BTR, branched-chain amino acids to tyrosine ratio; Alb, albumin; T-Bil, total-bilirubin; PT, prothrombin time; Na: serum sodium.

Table 4. Estimation of the usual daily nutritional condition using the FFQ

	Man (n = 22) Mean±SD	Woman (n = 19) Mean±SD	EAR or AI (man, woman)
Age (years)	68.5 ± 9.1	63.5 ± 13.3	
Height (cm)	166.0 ± 8.5	154.4 ± 4.1	
Body weight (kg)	63.9 ± 14.3	58.6 ± 11.9	
Ideal body weight (kg)	60.8 ± 6.1	52.5 ± 2.8	
BMI	23.0 ± 3.9	24.7 ± 5.6	
TEE (kcal)	1769.4 ± 309.3 *	1515.7 ± 149.7 **	
TEE / body weight (kcal/kg)	26.5 ± 1.6	28.1 ± 3.4	
<Results of FFQ>			
EIE（kcal/day）	1932.7 ± 533.4 *	1636.9 ± 455.8 **	2200-2650, 1700-2000
Ratio of EIE to EER	0.94 ± 0.29	0.93 ± 0.29	
EIE / body weight (kcal/kg)	30.9 ± 8.0	28.9 ± 8.5	
TEE / EIE	1.0 ± 0.5	1.0 ± 0.3	
Protein intake (g/kg)	1.2 ± 0.3	1.1 ± 0.4	

<Usual daily energy ratio>			
Protein (%)	15.1 ± 1.8	14.8 ± 2.5	20-30
Fat (%)	28.6 ± 5.2	30.2 ± 4.5	20-25
Carbohydrates (%)	56.6 ± 5.7	54.9 ± 6.2	50-70
Dietary fiber（g/day）	16.2 ± 6.0	16.2 ± 4.4	19<, 17<
n-3 USFA (g/day)	2.3 ± 1.0	1.8 ± 1.7	2.2<, 1.8<
n-6 USFA (g/day)	9.6 ± 3.9	8.9 ± 3.5	8-10, 7-9
n-6 USFA/n-3 USFA	4.5 ± 1.6	5.1 ± 1.4	
Salt (g/day)	9.8 ± 2.8	8.9 ± 2.9	9>, 7.5>

SD, standard deviation; TEE, total daily energy expenditure; EIE, estimated intake energy per day;
EER, estimated energy requirement; EAR, estimated average requirement;
n-3 USFA, n-3 unsaturated fatty acid; n-6 USFA, n-6 unsaturated fatty acid; AI, adequate intake;
*/**, There was no significant difference for the comparison of TEE and EIE; based on sex.

Table 5. The nutritional findings of patients with normal and abnormal digit symbol test results

Parameter	DST-Nor (n = 34) Mean±SD	DST-Abn (n = 7) Mean±SD	p value
Sex (Man:Wonam)	19 : 15	2 : 5	
<<Results of FFQ>>			
EIE（kcal/day）	1814.4 ± 553.7	1704.7 ± 136.4	p=0.826
Ratio of EIE to EER	0.94 ± 0.31	0.94 ± 0.16	p=0.852
EIE / body weight (kcal/kg)	29.8 ± 8.3	33.0 ± 8.2	p=0.466
Protein intake (g/kg)	1.1 ± 0.4	1.2 ± 0.4	p=0.834
<Usual daily energy ratio>			
Protein (%)	15.2 ± 2.0	13.8 ± 2.1	p=0.141
Fat (%)	29.3 ± 5.0	28.6 ± 4.2	p=0.533
Carbohydrates (%)	55.5 ± 6.1	57.7 ± 4.3	p=0.200
Dietary fiber（g/day）	16.5 ± 5.4	14.6 ± 3.2	p=0.703
n-3 USFA (g/day)	2.1 ± 0.9	1.9 ± 0.4	p=0.599
n-6 USFA (g/day)	9.4 ± 3.9	8.8 ± 2.2	p=0.795

n-6 USFA/n-3 USFA	4.8 ± 1.5	4.9 ± 1.5	p=0.876
Salt (g/day)	9.6 ± 2.9	8.4 ± 1.7	p=0.267

DST-Nor, normal digit symbol test; DST-Abn, abnormal digit symbol test;

EIE, estimated intake energy per day; EER, estimated energy requirements;

n-3 USFA, n-3 unsaturated fatty acid; n-6 USFA, n-6 unsaturated fatty acid.

23

24 Table 6. The clinical characteristics of LC patients that do not take BCAA supplementation
25 with normal and abnormal digit symbol test results

Characteristics	DST-Nor Mean ± SD or n (%)	DST-Abn Mean ± SD or n (%)	p value
Number of cases	25	7	
Age (years)	66.9 ± 10.9	61.7 ± 10.1	p = 0.266
Sex (M/F)	18 / 7	6 / 1	
BMI (kg/m^2)	24.2 ± 4.9	24.4 ± 3.4	p = 0.915
Number of cases with reduced muscle volume	1 (4.0)	2 (28.6)	p = 0.12
Child─Pugh score	5.9 ± 1.3	8.4 ± 3.6	p = 0.121
Child─Pugh classification (A/B/C)	21 / 3 / 1	3 / 1 / 3	
MELD score	7.6 ± 4.8	9.9 ± 7.3	p = 0.469
Complication			
HCC	18 (72.0)	2 (28.6)	p = 0.074
Ascites	5 (20.0)	3 (42.9)	p = 0.327
Concomitant medications			
Lactulose	0 (0.0)	0 (0.0)	
Antibiotics	0 (0.0)	0 (0.0)	
Zinc sulfate	1 (4.0)	0 (0.0)	p = 1.00
<Laboratory findings>			
ALT (U/L)	35.6 ± 25.1	46.6 ± 58.1	p = 0.641
Albumin (g/dL)	3.6 ± 0.5	3.0 ± 0.6	p = 0.05
Total-bilirubin (mg/dL)	1.0 ± 0.5	2.8 ± 2.0	p = 0.06
Platelet (×10^4/μL)	11.8 ± 5.9	15.9 ± 8.1	p = 0.251
Prothrombin time (%)	80.3 ± 13.3	69.4 ± 26.3	p = 0.326
Na (mmol/L)	140.2 ± 2.1	137.0 ± 4.7	p = 0.125
NH_3 （μg/dL）	52.3 ± 25.2	50.9 ± 16.6	p = 0.862
CRP (mg/dL)	0.31 ± 0.37	0.92 ± 0.77	p = 0.08

Amino Acids, standard value			
Valine (nmol/L), 158.4 ~ 287.7	222.6 ± 42.6	169.7 ± 77.6	p = 0.127
Leucine (nmol/L), 80.9 ~ 154.3	119.5 ± 27.9	93.5 ± 45.9	p = 0.195
Isoleucine (nmol/L), 41.3 ~ 84.9	69.4 ± 14.8	50.6 ± 24.3	p = 0.09
Methionine (nmol/L), 19.2 ~ 32.7	32.6 ± 9.3	29.2 ± 8.8	p = 0.403
Tyrosine (nmol/L), 50.2 ~ 82.6	100.2 ± 33.0	97.5 ± 26.0	p = 0.824
Phenylalanine (nmol/L), 45.7 ~ 76.5	84.6 ± 22.9	74.4 ± 21.0	p = 0.288
Histidine (nmol/L), 67.9 ~ 97.1	90.0 ± 13.3	73.8 ± 11.0	p = 0.00691
Tryptophan (nmol/L), 41.4 ~ 65.5	52.4 ± 15.5	43.0 ± 15.3	p = 0.185
Ornithine (nmol/L), 43.2 ~ 95.7	105.1 ± 29.9	81.3 ± 21.8	p = 0.036
Lysine (nmol/L), 118.7 ~ 257.0	188.7 ± 49.9	158.4 ± 39.3	p = 0.117
Arginine (nmol/L), 46.0 ~ 121.7	83.1 ± 26.2	57.2 ± 19.0	p = 0.012
BTR	4.58 ± 1.83	3.24 ± 1.21	p = 0.0385

DST-Nor, normal digit symbol test; DST-Abn, abnormal digit symbol test; BMI, body mass index; MELD, model for End-stage Liver Disease; HCC, hepatocellular carcinoma; BCAAs, branched-chain amino acids; ALT, alanine transaminase; Na, serum sodium; NH_3, serum ammonia; C-reactive protein; BTR, branched-chain amino acids to tyrosine ratio.