Usefulness of the Stroop Test in Diagnosing Minimal Hepatic Encephalopathy and Predicting Overt Hepatic Encephalopathy

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Minimal hepatic encephalopathy (MHE) adversely affects the clinical outcomes of patients with liver cirrhosis. This prospective study aimed to evaluate the utility of the Stroop test in the diagnosis of MHE and prediction of overt hepatic encephalopathy (OHE) in Japanese patients with cirrhosis. We enrolled 152 patients who underwent the Stroop test between November 2018 and February 2020. MHE was diagnosed using a combination of neuropsychological tests as the gold standard. The enrolled patients were followed up prospectively until the occurrence of OHE or August 2020. The optimal cutoff value of the Stroop test measurements was determined by receiver operating characteristic (ROC) curve analysis, and its predictive ability was assessed using the area under the ROC curve (AUC). Among the 139 eligible patients, 50 (36%) were diagnosed with MHE. The OffTime+OnTime cutoff value of 218.3 seconds had the best discriminative ability for MHE diagnosis, with an AUC of 0.77, a sensitivity of 74%, and a specificity of 75%. During a median follow-up of 10.8 months, 6 (4%) patients developed OHE. The OffTime+OnTime cutoff value of 305.6 seconds had the highest predictive ability for OHE, with an AUC of 0.79, a sensitivity of 67%, and a specificity of 92%. This value predicted OHE occurrence independent of liver functional reserve and prior OHE (hazard ratio, 19.8; *P* = 0.003). These two cutoff values remained statistically significant even when patients with prior OHE were excluded from the analysis. *Conclusion:* The Stroop test was useful for diagnosing patients with MHE and predicting OHE in Japanese patients with cirrhosis. (*Hepatology Communications* 2021;5:1518-1526).

H epatic encephalopathy (HE) is one of the most common complications of liver disease.[1-3] HE is characterized by a wide spectrum of neurological and psychiatric impairments, ranging from subclinical alterations to coma.[2] Minimal HE (MHE) is the mildest form in the spectrum of HE, and patients with MHE show no clinical signs or symptoms.[3] MHE is diagnosed using psychometric tests or neuropsychological tests (NPTs). Depending on the diagnostic criteria used and the population investigated, the prevalence of MHE in patients with cirrhosis varies from 20% to 80%.[2]

Recent evidence shows that MHE is associated with cognitive impairment, sarcopenia, poor health-related

quality of life, increased risk of falls, motor vehicle accidents, and hospitalization.[1-4] Moreover, MHE leads to negative clinical outcomes, such as overt hepatic encephalopathy (OHE) and mortality.[3,5,6] Some studies show that patients with HE grade 1 have a higher risk of mortality than those with no HE or MHE.[7] Several meta-analyses show that lactulose is effective in reversing MHE and preventing OHE occurrence in patients with cirrhosis.[8,9] Therefore, correctly, diagnosing MHE is important to predict adverse clinical outcomes in patients with cirrhosis and to provide timely treatments for patients with MHE who are at a high risk of OHE. Several tests that do not require psychological expertise and can be administered in a short time include the psychometric HE score, continuous reaction time, and critical flicker frequency.[2] However, diagnosis of MHE is still challenging and is seldom used in clinical practice because there are no typical symptoms and signs, no universally accepted diagnostic criteria, and need for financial resources.[2,10]

Tests for diagnosing MHE should be selected based on their ability to predict clinical outcomes and according to availability of reference data on the local population.[1] NPTs, which are standard psychometric tests, are used widely for the diagnosis of MHE in Japan[11-13] and other countries.[14-16] However, NPTs are time-consuming and require psychological expertise.[12] Therefore, it is difficult to perform these tests during routine clinical practice.

Recently, many studies have proposed tests that are simpler and more rapid than NPTs and that can be easily integrated into routine clinical practice.[10] The Stroop effect, which refers to the delay in reaction time between congruent and incongruent stimuli, is applied to psychological tests to assess cognitive and executive functioning.[16] The EncephalApp Stroop test, which uses the Stroop effect to evaluate psychomotor speed and cognitive flexibility, has attracted much attention as a reliable point-of-care test for diagnosing MHE.[1,2,10] Several studies have shown that this test has good reliability and external validity for the diagnosis of MHE,[17-20] although its clinical application in Japanese patients with cirrhosis is still unclear.

The aim of this prospective study was to clarify the relationship between the Stroop test and NPTs, to determine the optimal Stroop test cutoff score for diagnosing MHE in Japanese patients with cirrhosis, and to evaluate the ability of the test to predict OHE.

Materials and Methods

STUDY DESIGN

We recruited patients who were treated at Gifu University Hospital (Gifu, Japan) between November 2018 and February 2020. Observation time was defined as the time from enrollment to the last visit, the date of OHE occurrence, or August 31, 2020, whichever occurred first. The study objectives were explained to the patients by health care professionals not directly involved in their care, and written informed consent was obtained from all participants.

The study protocol was reviewed and approved by the institutional review board of the Gifu University Graduate School of Medicine (Approval No. 2018–164), and was in accordance with the 1975 Declaration of Helsinki. This prospective study was registered with the University Hospital Medical Information Network Clinical Trial Registry (No. UMIN000036632).

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STUDY SAMPLE

We recruited 152 patients with liver cirrhosis, aged between 20 and 79 years. The diagnosis of cirrhosis was based on laboratory variables, clinical features of portal hypertension, medical imaging, and/or histology if available. HE was diagnosed according to the West Haven criteria.[2] The Model for End-Stage Liver Disease (MELD) score,[21] not the MELD-Sodium score, was used to assess the severity of liver disease, because the MELD score is used commonly for evaluating liver functional reserve in Japanese patients with cirrhosis, and the indication for liver transplantation is determined based on this score.[22] All participants had a Mini-Mental State Examination score of >25 at enrollment. Mini-Mental State Examination is used commonly to diagnose dementia, and a score of >25 indicates that patients with cirrhosis are not prone to manifest the signs of HE.[23]

The exclusion criteria were refusal to provide informed consent, red-green color blindness, the presence of OHE at the time of screening, a history of organ transplantation, transjugular intrahepatic portosystemic shunting because of the increased risk of HE,[2] surgical shunt, uncontrolled hepatocellular carcinoma, gastrointestinal bleeding in the last 6 weeks, the presence of neurological or psychiatric diseases that may interfere with the MHE assessment, a history of brain injury or stroke, illicit drug use within the last 3 months, current use of psychoactive medication, nonhepatic active malignancy, and any acute life-threatening disease including severe sepsis, heart, respiratory, and/or renal failure.

NEUROPSYCHOLOGICAL TESTS

MHE was diagnosed using NPT software (v2.1) on an iPad (Apple Inc., Cupertino, CA). The software was developed by Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan) and was provided by the Japan Society of Hepatology. The NPT, which has been updated to take into account the aging population of patients with cirrhosis,[24] consists of four subtests including the number connection test (NCT)-A (time taken to connect numbers only), the NCT-B (time taken to connect numbers and letters alternately), the digit symbol test (DST) (total number of special symbols and numbers matched correctly within 60 seconds), and the block design test (BDT) (time taken to duplicate a standardized design using given blocks).[16] Short times on the NCT-A, NCT-B and BDT, and high DST scores indicate good cognitive performance. Patients were diagnosed with MHE if the results of two or more of the four subtests were below the reference range.[10]

STROOP TEST

The Stroop test was administered according to the method described in the literature.[17-19] We used the translated Japanese version of the EncephalApp Stroop test in this study. After completing the NPT, the participants took the Stroop test on an iPad. The Stroop test consists of "Off" and "On" states, depending on the concordance or discordance of the stimuli. Before the test, two training runs were conducted to familiarize the participants with the test. The Stroop test measurements included the time taken to complete five runs correctly in the "Off" (OffTime) and "On" (OnTime) states, total time (OffTime+OnTime), extra time in the "On" state (OnTime-OffTime), and number of runs required to complete five correct runs in the "Off" and "On" states.[17]

STATISTICS

Continuous variables were tested for normality using the Shapiro–Wilk test. Data were expressed as the number of patients and percentage (%) for categorical variables and as median and interquartile range for continuous variables. The chi-square test for categorical variables and Mann-Whitney U test for continuous variables were used to compare the two groups. Spearman's rank correlation coefficient was used to determine the relationships between variables. Receiver operating characteristic (ROC) curve analysis was used to assess the discriminative ability of the Stroop test measurements, and the results were presented as the area under the curve (AUC). The optimal cutoff value was estimated using the highest Youden index value. Predictors of OHE development were analyzed using Cox proportional hazards models, and the results were presented as hazard ratios (HRs) with 95% confidence intervals (95% CIs). The cumulative incidence curves were estimated using the Kaplan-Meier curve and compared between the two groups using the log-rank test. The significance threshold was set at $P < 0.05$.

All analyses were performed using JMP version 9.0.2 software (SAS Institute Inc., Cary, NC).

Results

PATIENT CHARACTERISTICS

Among the initial cohort of 152 patients, 13 were excluded from the study: 7 for lack of data on the NPT and Stroop test, 4 for lack of informed consent, and 2 for color-blindness. The baseline characteristics of the remaining 139 patients are given in Table 1. Among the analyzed patients, 77 (55%) were men, and the median age and MELD score were 70 years and 8, respectively. A history of OHE was reported by 9 patients, but the disease was well controlled using lactulose and/or rifaximin. The baseline characteristics of patients without a history of OHE are provided in Supporting Table S1.

MHE was diagnosed in 50 (36%) patients using NPT as the gold standard. The MHE group had more patients with a history of OHE, a higher prevalence of esophagogastric varices, and higher ammonia and lower albumin levels than the non-MHE group. In addition, using the international normalized ratio and MELD score, all of these factors indicated more severe liver disease, and tended to be higher in the MHE group (Table 1).

TABLE 1. BASELINE CHARACTERISTICS OF PATIENTS WITH AND WITHOUT MHE

	Total Cohort	No MHE	MHE	P Value*
Characteristics	(n = 139)	(n = 89)	(n = 50)	
Age (years)	70 (61-76)	70 (60-76)	68 (62-76)	0.72
Male sex	77 (55)	47 (53)	30 (60)	0.48
Etiology				
Virus/alcohol/others	46/25/68	25/18/46	21/7/22	0.23
Hepatocellular carcinoma	56 (40)	35 (39)	21 (42)	0.86
Prior OHE	9 (7)	3 (3)	6 (12)	0.047
Esophagogastric varices	79 (57)	44 (49)	35 (70)	0.021
MELD score†	8 (7-11)	8 (7-9)	9 (7-11)	0.06
Total bilirubin (mg/dL)	1.0 (0.7-1.5)	1.0 (0.7-1.4)	1.1 (0.8-1.5)	0.35
Creatinine (mg/dL)	0.70 (0.58-0.88)	0.70 (0.60-0.86)	0.70 (0.55-0.89)	0.66
International normalized ratio	1.07 (1.00-1.21)	1.04 (1-1.17)	1.11 (1.02-1.25)	0.07
Albumin (g/dL)	4.0 (3.6-4.3)	4.1 (3.6-4.3)	3.7 (3.4-4.1)	0.014
Sodium (mEq/L)	139 (137-140)	139 (137-140)	139 (137-141)	0.74
Ammonia (µg/dL)	50 (37-72)	47 (34-68)	53 (39-97)	0.043
Zinc (µg/dL)	71 (58-80)	74 (62-81)	67 (56-77)	0.13
NPT results				
NCT-A (seconds)	47.6 (35.8-68.5)	41.4 (32.6-53.6)	67.6 (47.1-84.5)	<0.001
NCT-B (seconds)	75.9 (57.0-104.0)	66.8 (51.6-82.3)	98.8 (79.1-143.0)	<0.001
DST (raw score)	14 (10-18)	16 (12-19)	11 (8-15)	<0.001
BDT (seconds)	77.1 (57.0-103.3)	65.1 (53.4-89.8)	101.2 (84.0-131.0)	<0.001
Stroop test results				
OffTime (seconds)	96.6 (83.5-115.6)	88.5 (80.9-100.5)	112.7 (97.9-138.2)	<0.001
OnTime (seconds)	108.2 (94.0-131.5)	101.8 (91.1-116.2)	132.2 (112.1-150.1)	<0.001
Off state runs	5 (5-5)	5 (5-5)	5 (5-5)	0.14
On state runs	5 (5-5)	5 (5-5)	5 (5-5)	0.14
OffTime+OnTime (seconds)	203.2 (176.8-246.4)	190.1 (169.8-216.6)	244.5 (212.2-288.3)	<0.001
OnTime-OffTime (seconds)	13.5 (5.7-21.1)	12.0 (5.4-18.4)	18.1 (8.7-28.9)	0.011

Note: Values are presented as number (percentage) or median (interquartile range).
*The chi-square test for categorical variables or Mann-Whitney U test for continuous variables was used to compare the clinical characteristics between the two groups.
†The severity of liver disease was assessed using the MELD score, not MELD-Sodium score.

TABLE 2. AUC VALUES FOR THE STROOP TEST

Characteristics	AUC (95% CI)	Cutoff value	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)
Stroop test results					
OffTime (seconds)	0.75 (0.67-0.84)	97.8	0.76 (0.65-0.85)	0.69 (0.63-0.73)	0.71 (0.64-0.77)
OnTime (seconds)	0.76 (0.67-0.85)	124.8	0.64 (0.54-0.73)	0.82 (0.76-0.87)	0.76 (0.68-0.82)
Off state runs	0.54 (0.48-0.60)	6	0.15 (0.08-0.21)	0.93 (0.89-0.96)	0.65 (0.61-0.70)
On state runs	0.54 (0.48-0.60)	6	0.15 (0.08-0.21)	0.93 (0.89-0.96)	0.65 (0.61-0.70)
OffTime+OnTime (seconds)	0.77 (0.68-0.85)	218.3	0.74 (0.64-0.83)	0.75 (0.69-0.80)	0.75 (0.67-0.81)
OnTime-OffTime (seconds)	0.63 (0.52-0.74)	15.1	0.62 (0.51-0.72)	0.66 (0.60-0.72)	0.65 (0.57-0.72)

STROOP TEST FOR MHE DIAGNOSIS

Patients with MHE showed poor cognitive function on the Stroop test, except for the "Off" and "On" state runs (Table 1). ROC curve analysis showed that the OffTime+OnTime cutoff value of 218.3 seconds had the highest discriminative ability (AUC, 0.77; 95% CI, 0.68-0.85; sensitivity, 0.74; specificity, 0.75; accuracy, 0.75) to diagnose MHE, followed by OnTime, OffTime, and OnTime-OffTime (Table 2). Similarly, the OffTime+OnTime cutoff value (218.3 seconds) had the highest AUC (AUC, 0.75; 95% CI, 0.66-0.84), even when patients with a history of OHE were excluded (Supporting Table S2).

The enrolled patients were classified into two groups based on the OffTime+OnTime cutoff value of 218.3 seconds, and 59 patients (42%) who obtained scores higher than the cutoff value were diagnosed with MHE (Table 3). Patients with OffTime+OnTime ≥ 218.3 seconds were older, had a higher frequency of prior OHE, a higher prevalence of esophagogastric varices, and lower albumin and zinc levels than those with OffTime+OnTime < 218.3 seconds. Patients with OffTime+OnTime ≥ 218.3 seconds showed poor performance on all subtests of the NPT (all, $P < 0.001$). OffTime+OnTime was significantly correlated with NCT-B (r = 0.67; $P < 0.001$), DST (r = –0.76; $P < 0.001$), and BDT (r = 0.76; all $P < 0.001$), whereas the correlation between OffTime+OnTime and NCT-A was relatively low (r = 0.50; $P < 0.001$). Similar results were obtained when patients with a history of OHE were excluded from the analysis (Supporting Table S3).

STROOP TEST FOR OHE PREDICTION

During the follow-up period (median, 10.8 months; interquartile range, 5.8-13), 6 (4%) patients experienced grade 2 or higher HE. No patient died or underwent liver transplantation during the study period. The Cox proportional hazards model showed that a history of OHE (HR, 11.09; 95% CI, 2.02-60.94; $P = 0.006$) and MELD score (HR, 1.25; 95% CI, 1.01-1.54; $P = 0.039$) were significantly associated with an increased risk of OHE occurrence, whereas increased albumin level was a protective factor (HR, 0.17; 95% CI, 0.05-0.59; $P = 0.005$). All NPT and Stroop test results, except for OnTime-OffTime, were significantly associated with the development of OHE (Supporting Table S4). Similar results were obtained when patients with a history of OHE were excluded (Supporting Table S5).

ROC curve analysis showed that the OffTime+OnTime cutoff value of 305.6 seconds had the highest predictive ability for OHE occurrence (AUC, 0.79; 95% CI, 0.53-1.00; sensitivity, 0.67; specificity, 0.92; accuracy, 0.91). Multivariate analysis showed that OffTime+OnTime ≥ 305.6 seconds was a significant predictor of OHE occurrence (HR, 19.81; 95% CI, 2.84-170.21; $P = 0.003$), independent of MELD score and history of OHE (Supporting Table S6). This cutoff value remained statistically significant when patients with a history of OHE were excluded from the analysis (HR, 16.93; 95% CI, 2.00-146.25; $P = 0.013$) (Supporting Table S7).

CUMULATIVE INCIDENCE OF OHE

When MHE was diagnosed using NPT, OHE was found in 8% (4 of 50) of the patients with MHE and 2% (2 of 89) of those without MHE ($P = 0.11$). The difference in cumulative incidence of OHE between patients with and without MHE diagnosed using NPT was not statistically significant ($P = 0.09$; Fig. 1A). When MHE was diagnosed using the OffTime+OnTime cutoff value of 218.3 seconds, OHE was found in 8% (5 of 59) of the patients with MHE

TABLE 3. COMPARISON OF BASELINE CHARACTERISTICS OF PATIENTS WITH OFFTIME+ONTIME < 218.3 SECONDS AND THOSE WITH ≥218.3 SECONDS

Characteristics	OffTime+OnTime < 218.3 Seconds (n = 80)	OffTime+OnTime ≥ 218.3 Seconds (n = 59)	P Value*
Age (years)	66 (58-71)	75 (69-77)	<0.001
Male sex	43 (54)	34 (58)	0.73
Etiology			
Virus/alcohol/others	24/16/40	22/9/28	0.60
Hepatocellular carcinoma	29 (36)	27 (46)	0.30
Prior OHE	1 (1)	8 (14)	0.005
Esophagogastric varices	32 (40)	47 (80)	<0.001
MELD score†	8 (7-9)	8 (7-11)	0.28
Total bilirubin (mg/dL)	1.0 (0.7-1.5)	1.0 (0.6-1.4)	0.35
Creatinine (mg/dL)	0.68 (0.58-0.84)	0.73 (0.60-0.90)	0.34
International normalized ratio	1.04 (1.00-1.20)	1.08 (1.02-1.22)	0.31
Albumin (g/dL)	4.1 (3.6-4.4)	3.8 (3.5-4.1)	0.031
Sodium (mEq/L)	139 (137-140)	139 (137-141)	0.33
Ammonia (μg/dL)	49 (37-63)	51 (37-84)	0.16
Zinc (μg/dL)	75 (64-83)	65 (56-78)	0.026
NPT results			
NCT-A (seconds)	41.5 (32.6-53.1)	63.1 (43.2-84.0)	<0.001
NCT-B (seconds)	66.7 (51.7-81.6)	106.0 (72.5-140.4)	<0.001
DST (raw score)	17 (14-20)	10 (8-12)	<0.001
BDT (seconds)	62.2 (51.2-76.0)	106.0 (88.8-130.8)	<0.001
MHE on NPT	13 (16)	37 (63)	<0.001

Note: Values are presented as number (percentage) or median (interquartile range).
*The chi-square test for categorical variables or Mann-Whitney U test for continuous variables was used to compare the clinical characteristics between the two groups.
†The severity of liver disease was assessed using the MELD score, not MELD-Sodium score.
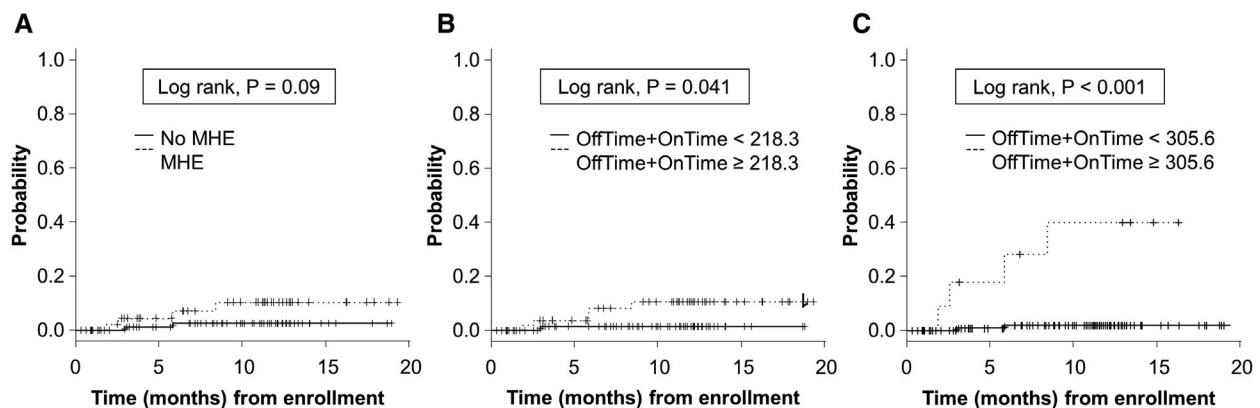
FIG. 1. Cumulative incidence of OHE in different subgroups. (A) Patients with and without MHE, based on NPT results. (B) Patients with OffTime+OnTime < 218.3 seconds and those with OffTime+OnTime ≥ 218.3 seconds. (C) Patients with OffTime+OnTime < 305.6 seconds and those with OffTime+OnTime ≥ 305.6 seconds. Probability was estimated using the Kaplan-Meier method and compared between groups using the log-rank test.

and 1% (1/80) of those without MHE (P = 0.038). According to the diagnostic criteria determined by the Stroop test, the cumulative incidence of OHE at 12 months was significantly higher in patients with MHE than in those without MHE (Kaplan-Meier estimates: 11% vs. 1%; P = 0.041; Fig. 1B), with an HR of 6.9 (95% CI, 1.11-131.53; P = 0.038). Additionally, OHE was found in 27% (4 of 15) of the patients with OffTime+OnTime ≥ 305.6 seconds and 2% (2 of 124) of those with OffTime+OnTime < 305.6 seconds (P < 0.001). At 12 months, patients with OffTime+OnTime ≥ 305.6 seconds had a significantly higher cumulative incidence of OHE than those with OffTime+OnTime < 305.6 seconds (Kaplan-Meier estimates: 40% vs. 2%; P < 0.001; Fig. 1C), with an HR of 23.8 (95% CI, 4.63-171.49; P < 0.001).

Discussion

This prospective study was designed to investigate the utility of the Stroop test in diagnosing MHE and predicting OHE in Japanese patients with cirrhosis. Because MHE adversely affects clinical outcomes in patients with cirrhosis, there is an unmet clinical need for simple and convenient methods to identify patients who are at a high risk of MHE. Although various methods have been developed and introduced to diagnose MHE, there is little evidence on point-of-care diagnostic tests that can be easily implemented in routine clinical practice.[10] The Stroop test is a valid and reliable test for diagnosing MHE.[17-19] Additionally, it is a time-saving and user-friendly method with good accessibility, convenience, and acceptability, all of which may lead to improved MHE diagnosis rates.[19] However, its applicability in the Japanese context remains unclear. This study confirms previous findings[5,17,18] and provides evidence that the Stroop test has a high discriminative ability with regard to MHE diagnosis and predictive ability with regard to OHE occurrence in Japanese patients with cirrhosis.

Recent evidence has shown that OffTime+OnTime has an advantage over other Stroop test results in its ability to distinguish patients with and without MHE, but there is no consensus on its optimal cutoff value.[1,16] This study found that the OffTime+OnTime cutoff value of 218.3 seconds had the best discriminative ability for the diagnosis of

MHE, whereas other studies have shown various cutoff values ranging from 187 to 275 seconds.[16-19,25] The reason behind this difference is unclear, but the predominance of older participants (average 70 years) in our study may have influenced our results.[17,20] Because the cutoff value for MHE diagnosis should be based on specific clinical circumstances,[1] we first identified the optimal value for MHE diagnosis in Japanese patients with cirrhosis.

Using the Stroop test, we found that old age, history of OHE, and presence of esophagogastric varices were associated with poor cognitive function. These findings corroborate those of previous studies that evaluated the relationship between the Stroop test and MHE.[17-19] Because it is widely accepted that cognitive function declines with age, the relationship between poor performance on the Stroop test and age is biologically plausible.[26] Several studies have shown that patients with a history of OHE require a longer time to complete the Stroop test, suggesting that prior OHE, even under well-controlled conditions, affects the results of cognitive tests.[3,17] The reason behind the association between esophagogastric varices and impaired cognitive function can be explained by the fact that esophagogastric varices are an indicator of portal hypertension, leading to increase the risk of HE.[27,28]

Our study provides evidence that the Stroop test is superior to NPT in the prediction of OHE in patients with cirrhosis. However, these results should be interpreted with caution, because the data analysis relies on the low number of OHE events. The findings of our study complement those of previous studies. For instance, it has been reported that the Stroop test can predict OHE occurrence independently of the MELD score and history of OHE.[18] The Stroop test alone has a higher accuracy than the two-test combination in predicting OHE.[5] However, the two aforementioned studies used a formula that included age, sex, and education in the calculation of OffTime+OnTime, which limits its implementation and usefulness in clinical practice. Our study identified clear cutoff values for diagnosing MHE and predicting OHE; hence, we believe our findings have meaningful clinical implications for daily clinical practice. Moreover, the simplicity and usefulness of the Stroop test in the diagnosis of MHE may contribute to the timely treatment of patients with MHE who are at a high risk of OHE.

This study has several limitations. First, our sample size was small, and there was no information on

Japanese reference values and no external validation of the Stroop test. Second, this study cannot rule out the possibility that education has an impact on cognitive functioning. For example, education that reflects familiarity with app-based testing may affect the results of cognitive function tests and consequently lead to selection bias.[19] Some studies on the Stroop test have shown that higher education is associated with improved cognitive function in patients with cirrhosis,[19,20] whereas other studies did not find such a relationship.[16, 17] Because the expected number of years of schooling in Japan is relatively high (15.2 years),[29] we infer that education level had little impact on the results of this study. However, further research is needed to clarify the normal reference values for Japanese patients adjusted for age and education level, as not all Japanese patients have the same level of education. Third, the low MELD score of the enrolled patients could explain the low number of OHE events during the follow-up period, thus limiting our conclusions about the predictive ability of the Stroop test. Fourth, because the cutoff values for the diagnosis of MHE and prediction of OHE were obtained from the Japanese cohort, these finding may not be generalized to other cohorts. Finally, because patients with cirrhosis are elderly, have several comorbidities, and are taking psychotropic medications,[2,10] our findings may not be applicable to all clinical settings. Given these limitations, large, long-term, multicenter, prospective studies are required to determine whether our findings can be translated into daily clinical practice involving other populations and regions.

In conclusion, this prospective study provides substantial evidence that the Stroop test is useful for diagnosing MHE and predicting OHE in Japanese patients with cirrhosis. It may be premature to reach such conclusions, and there may be other possible interpretations of our findings. However, this study can be considered as one of the initial steps toward the improvement of strategies for the diagnosis of MHE.

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