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Original Article

Evaluation of Plasma Neurodegenerative Biomarkers for Diagnosing Minimal Hepatic Encephalopathy and Predicting Overt Hepatic Encephalopathy in Chinese Patients with Hepatic Cirrhosis

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Abstract

Background and Aims: The performance of neurodegenerative biomarkers—neurofilament light chain (NfL), glial fibrillary acidic protein (GFAP), tau, and ubiquitin carboxy-terminal hydrolase L1 (UCHL1)—in diagnosing minimal hepatic encephalopathy (MHE) has not been systematically evaluated, simultaneously, nor have their associations with the development of overt hepatic encephalopathy (OHE). This study aimed to evaluate the performance of plasma NfL, GFAP, tau, and UCHL1 in diagnosing MHE and predicting the development of OHE in Chinese patients with hepatic cirrhosis. ***Methods:*** In this prospective study, 124 patients with hepatic cirrhosis were recruited. The Psychometric Hepatic Encephalopathy Score was used to diagnose MHE, and OHE development was observed during a 30-day follow-up period. Plasma levels of NfL, GFAP, tau, and UCHL1 were measured using the highly sensitive single-molecule array when MHE was diagnosed. Additionally, serum interleukin-6 (IL-6) levels and the model for end-stage liver disease (MELD) and MELD-Na scores were also measured. ***Results:*** MHE was diagnosed in 57 (46.0%) patients. Patients with MHE had significantly higher plasma levels of NfL and GFAP (34.2 *vs*. 22.4 pg/mL and 173 *vs*. 97.6 pg/mL, respectively; both $p < 0.001$) and lower tau levels (8.4 *vs*. 11.6 pg/mL, $p = 0.048$) compared to those without MHE. Plasma NfL (odds ratios = 1.027, 95% confidence interval [CI]: 1.006–1.048; $p = 0.013$) and serum ammonia levels (odds ratios = 1.021, 95% CI: 1.006–1.036; $p = 0.007$) were independently associated with MHE occurrence. A combination of NfL, GFAP, tau, and UCHL1 was effective in diagnosing MHE in all cirrhotic patients (area under the receiver operating characteristic curve [hereinafter referred to as AUROC]: 0.748, 95% CI: 0.662–0.821), with an accuracy, sensitivity, and specificity of 71.0%, 71.9%, and 71.6%, respectively. In patients without previous OHE, the combination had an AUROC of 0.764 (95% CI: 0.673–0.840), with an accuracy, sensitivity, and specificity of 72.5%, 71.7%, and 73.0%, respectively. Furthermore, GFAP (hazard ratio (HR) = 1.003, 95% CI: 1.000–1.005; $p = 0.044$), IL-6 (HR = 1.003, 95% CI: 1.001–1.004; $p < 0.001$), and MELD score (HR = 1.139, 95% CI: 1.072–1.210; $p < 0.001$)—but not NfL, tau, and UCHL1—were identified as risk factors for 30-day OHE development. ***Conclusions:*** The combination of plasma levels of NfL, GFAP, tau, and UCHL1 performs well in diagnosing MHE. Additionally, MELD score, IL-6, and GFAP appear to be significant predictors of OHE development in patients with hepatic cirrhosis.

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Introduction

Liver cirrhosis is a globally prevalent condition that typically progresses from a latent, asymptomatic state, known as compensated cirrhosis, to a more advanced, symptomatic stage called decompensated cirrhosis. Complications developing in the decompensated phase frequently lead to hospitalization, significantly diminish the patient's quality of life, and contribute to a high mortality rate.[1] Hepatic encephalopathy (HE) is among the most severe complications associated with liver cirrhosis, characterized by a broad range of neurological and psychiatric symptoms. These can vary from subtle, sub-

Keywords: Minimal hepatic encephalopathy; Overt hepatic encephalopathy; Neurofilament light chain; Glial fibrillary acidic protein; Tau; Ubiquitin carboxy-terminal hydrolase L1.

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clinical changes to severe impairments such as coma.[2] The severity of HE is graded on a scale of 0–4 in terms of mental status according to the West Haven Criteria.[3,4] Grades 0–1 are classified as minimal HE (MHE), while a grade of 1 HE along with MHE belongs to covert HE, and grades 2–4 are categorized as overt HE (OHE).[4] MHE, whose prevalence in patients with cirrhosis varies from 25% to 52% depending on disease stages,[5] despite its lack of discernible clinical evidence, is associated with abnormalities in patients' daily cognition, emotion, muscular strength, driving ability, quality of life, and socioeconomic status.[6–8] More importantly, MHE can progress to OHE,[9] which is easily recognized by obvious clinical symptoms such as disorientation, somnolence, and a coma-like state, consequently increasing the risk of mortality and poor prognosis.[4,6] Therefore, the diagnosis of MHE and identification of the predictive factors for the development of OHE are essential.

Several psychometric tests, including the psychometric hepatic encephalopathy score (PHES), animal naming test, EncephalApp, as well as their modified versions, such as simplified animal naming test, QuickStroop, and advanced magnetic resonance imaging (MRI) techniques, have been used for diagnosing MHE.[5,10–13] The PHES test is the most frequently used and widely considered the gold standard.[4] However, there is no universal agreement on the reference values for these tests, largely due to cultural and geographical variations in the diagnosis of MHE.[14,15] Moreover, these tests are not feasible in daily clinical practice because they are time-consuming or inconvenient for hospitalized patients with severe illness.[16] Recently, a few time-saving and convenient blood biomarker tests have been evaluated. For example, the serum ammonia level is widely used in HE diagnosis due to its high negative predictive value; however, its accuracy in recognizing MHE remains controversial.[16]

Previous studies have revealed that astrocytic swelling, astrocytic and neuronal dysfunction, profound mitochondrial dysfunction, and severe injury or death of neuronal cells contribute to HE symptoms.[3,4,17] Microglia activation, synaptic over-pruning, astrocytic neurodegeneration, and systemic inflammation-altered neurotransmission are associated with MHE pathogenesis.[18–20] Recently, several serum neurodegenerative biomarkers have been explored in the diagnosis of neurological damage-related diseases and complications.[21–24] Neurofilament light chain (NfL), glial fibrillary acidic protein (GFAP), tau, and ubiquitin carboxy-terminal hydrolase L1 (UCHL1) are among these biomarkers. It has been reported that increased serum levels of NfL and GFAP are independently associated with the presence of MHE in patients with hepatic cirrhosis.[25,26] However, their performance in diagnosing MHE has not been systematically evaluated simultaneously, nor have their associations with the development of OHE in Chinese patients with hepatic cirrhosis. In addition, there are no reports evaluating the associations of tau and UCHL1 with MHE. Moreover, the potential value of these four biomarkers in predicting the development of OHE has never been explored.

Therefore, the present prospective study was carried out to determine the performance of plasma levels of NfL, GFAP, tau, and UCHL1 in diagnosing MHE and predicting OHE development in patients with hepatic cirrhosis.

Methods

Study design and participants

In the present prospective cohort study, a consecutively recruited cohort of hospitalized patients with hepatic cirrhosis between 2019 and 2021 at Tongji Hospital, Wuhan, China, was included. The inclusion criteria were as follows: (1) age > 18 years and (2) diagnosis of hepatic cirrhosis based on histology, abdominal imaging, and medical history.[1] The exclusion criteria were as follows: (1) status of OHE and an episode of OHE within the past six weeks; (2) concomitant nervous system diseases, such as dementia or stroke; (3) a history of recent head trauma or surgery; (4) organic lesions in the brain, such as hemorrhages or infarction; (5) inability to complete the PHES; (6) a history of transjugular intrahepatic portosystemic shunt or abdominal imaging demonstrating portosystemic shunts within the past three months; (7) high alcohol consumption (>30 g/day in men or >20 g/day in women)[27] or psychoactive medication use (*e.g.*, benzodiazepines, neuroleptics, antiepileptics, and opiates) in the past four weeks; (8) incomplete essential clinical data; and (9) unavailability of plasma samples collected at baseline.

Diagnostic criteria of MHE

PHES, utilized as the gold standard for detecting MHE, was employed in this study. Patients with a total score below −4, based on normalization criteria adjusted for age and education, were diagnosed with MHE.[28–30] All psychometric and neuropsychological testing procedures were consistently administered by the same experienced clinician to the patients after admission, in a quiet environment between 9 a.m. and 4 p.m., to minimize the impact of confounding factors.

Collection of demographic and clinical data

At admission, patients' demographic and socioeconomic information, including age, sex, education level, job, and medical history (e.g., previous history of OHE and other diseases), were recorded. Moreover, data on laboratory findings such as serum ammonia, interleukin-6 (IL-6), C-reactive protein (CRP), and procalcitonin (PCT), pathological data such as the model for end-stage liver disease (MELD) score, imaging examinations such as ultrasound, computed tomography, and MRI, and interventions for HE such as L-ornithine L-aspartic acid, lactulose, and probiotics,[3,4] during hospitalization, were prospectively collected and entered into the medical record system. Data on the length of hospitalization and hepatic cirrhosis-related complications, including ascites, pleural effusion, portal hypertension, esophageal and gastric varices, gastrointestinal hemorrhage, spontaneous bacterial peritonitis, pulmonary infection, urinary system infection, fungal infection, and other infections (e.g., cytomegalovirus and Epstein-Barr virus), were also collected.

Quantification of plasma NfL, GFAP, tau, and UCHL1 levels

Blood samples were collected *via* venipuncture into tubes containing ethylene diamine tetraacetic acid on the day of neuropsychological testing. Plasma was aliquoted after centrifugation (2000 g, 4°C, 10 m) within 60 m and stored in cryotubes at −80°C. Plasma levels of NfL, GFAP, tau, and UCHL1 were measured in the same batch using a single-molecule array (SiMoA Human Neurology 4-Plex A; Quanterix) platform by a laboratory technician blinded to the patient's clinical data.

Follow-up evaluation

All patients were followed for 30 days from enrollment or until an episode of OHE through regular clinic visits to the center or via telephone review. Each patient was evaluated by an experienced hepatologist for the episode and degree, if any, of OHE according to the West Haven criteria.[2] An OHE

episode during the 30-day follow-up period was defined as 30-day OHE development.

Ethical statement

The study protocol was approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (S196). Informed consent was obtained from all patients.

Statistical analysis

Categorical variables are presented as frequencies and percentages. Quantitative values for neurodegenerative biomarkers and other measurements are reported as the mean ± standard deviation or median with interquartile range (IQR), depending on the data distribution. An independent samples t-test or the nonparametric Mann-Whitney U test was employed to assess differences in biomarker levels between patients with MHE and those without. Boxplots depicting the median, IQR, and range were utilized to illustrate biomarker variations. Correlation analysis was conducted using the R package corrplot. The chi-square test was applied to compare categorical variables between the two groups, with odds ratios (ORs) and 95% confidence intervals (CIs) calculated. Multivariate logistic regression analysis, including variables with a *p*-value less than 0.1 in the univariate analysis, was conducted to identify independent factors associated with MHE. To ascertain the diagnostic accuracy of neurodegenerative biomarkers in MHE, we analyzed metrics including the area under the receiver operating characteristic curve (AUROC), sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV). Spearman's rank correlation coefficients were calculated to evaluate the relationships between biomarker levels and demographic factors, naming tests, and clinical parameters in cirrhotic patients without a prior episode of OHE.

Furthermore, multivariate Cox logistic regression analysis was performed to investigate potential risk factors for 30-day OHE development, assessing the hazard ratios (HRs) of neurodegenerative biomarkers, cirrhosis-associated complications, and other factors.

The threshold for statistical significance was set at a two-tailed *p*-value less than 0.05. The analyses were conducted using statistical software packages, including SPSS version 26.0 (SPSS Inc., Chicago, IL, USA), GraphPad Prism version 8.0.1 (GraphPad Software, La Jolla, CA, USA), MedCalc version 11.4.2.0 (MedCalc Software, Ostend, Belgium), and R version 4.1.3 (R Core Team, 2021, The R Foundation for Statistical Computing, Vienna, Austria).

Results

Demographic and clinical characteristics of patients with hepatic cirrhosis

A total of 337 patients with hepatic cirrhosis were screened, and 119 patients were excluded due to OHE status and previous OHE within the last six weeks (n = 21), cerebral infarction (n = 3), vision disorders (n = 3), recent consumption of alcohol or psychoactive drugs (n = 10), portosystemic shunts (n = 1), incomplete essential clinical data (n = 27), and unavailable plasma samples collected at admission (n = 54). Thus, 124 patients with hepatic cirrhosis (95 males, 29 females; age 54 [47; 62]) were included in the present study (Fig. 1 and Table 1). Hepatitis related to hepatic viruses was the most common underlying etiology for hepatic cirrhosis, accounting for 75.8% (n = 94), followed by chronic alcohol-related cirrhosis (3.2%, n = 4), autoimmune (3.2%, n = 4), cholestatic (1.6%, n = 2), schistosomiasis (2.4%, n = 3), and others (drug-induced/metabolic/unknown)(13.7%, n = 17). The medians of MELD and MELD-Na scores in all patients were 16 (IQR 9–21) and 18 (IQR 13–25), respectively (Table 1). Of the 124 patients, 15 (12.1%) had a history of OHE, and 57 (46.0%) were diagnosed with MHE according to the PHES test. Additionally, OHE developed in 18 (14.5%) patients within 30 days after the PHES test (Table 1).

Plasma NfL, GFAP, tau, and UCHL1 levels in cirrhotic patients

The plasma levels of NfL, GFAP, tau, and UCHL1 in all studied patients with hepatic cirrhosis were 28.1 pg/mL [IQR 19.3; 48.2], 144 pg/mL [80.2; 203.4], 10.4 pg/mL [6.4; 17.9], and 48.7 pg/mL [34.9; 91.6], respectively (Table 1). Among patients without previous OHE, elevated NfL and GFAP levels were positively correlated with older age, MHE, and serum IL-6 levels (Table 2). There were significant negative correlations between the levels of plasma NfL ($r = -0.195$, $p = 0.045$) and UCHL1 ($r = -0.219$, $p = 0.024$) and decreased levels of serum albumin (Table 2). Plasma levels of tau ($r = 0.276$, $p = 0.004$) and UCHL1 ($r = 0.249$, $p = 0.009$) were associated with delayed hospitalization, but only tau was correlated with MELD ($r = 0.236$, $p = 0.017$) and MELD-Na ($r = 0.332$, $p = 0.001$). There were no correlations between these four neurodegenerative biomarkers and PCT, CRP, hepatic virus-related cirrhosis, or hepatic cirrhosis-related complications, except for tau ($r = 0.221$, $p < 0.01$) with pleural effusion, UCHL1 with ascites ($r = 0.282$, $p = 0.003$), and GFAP with esophageal and gastric varices ($r = -0.196$, $p = 0.041$) (Table 2).

Plasma NfL, GFAP, tau, and UCHL1 levels in patients with and without MHE

Plasma NfL and GFAP levels in patients with MHE were significantly higher than in those without MHE (NfL 34.2 pg/mL [IQR 26.9; 78.8] *vs.* 22.4 pg/mL [IQR 16.5; 35.5], $p < 0.001$; GFAP 173 pg/mL [IQR 126.2; 239.4] *vs*. 97.6 pg/mL [IQR 68.2; 178.8], $p < 0.001$, Table 1 and Fig. 2A, B). Plasma tau levels in patients with MHE were significantly lower than in those without MHE (8.4 pg/mL [IQR 5.6; 17.2] *vs*. 11.6 pg/mL [IQR 8.1; 19.2], $p = 0.048$, Table 1 and Fig. 2C**).** Plasma UCHL1 levels did not significantly differ between patients with and without MHE (49.1 pg/mL [IQR 32.9; 81.6] *vs*. 48.2 pg/mL [IQR 37.3; 98.1], $p = 0.546$, Table 1 and Fig. 2D).

Based on the univariable analysis (Table 1), plasma NfL, GFAP, tau levels, age, serum ammonia, and previous OHE were included in the multivariable logistic regression model. In this model, NfL levels were significantly associated with MHE, with an OR of 1.027 (95% CI 1.006–1.048; $p = 0.013$; Table 3). Moreover, NfL levels remained significantly associated with MHE (OR 1.024; 95% CI 1.003–1.045; $p = 0.024$, Table 3) when patients with previous OHE were excluded from the analysis.

Diagnostic performance of plasma NfL, GFAP, tau, and UCHL1 levels for MHE

Plasma NfL and GFAP levels distinguished patients with MHE with an AUROC of 0.719 (95% CI: 0.632–0.796) and 0.690 (95% CI: 0.600–0.770), respectively. The AUROCs for tau and UCHL1 levels distinguishing patients with MHE were 0.603 (95% CI: 0.511–0.689) and 0.532 (95% CI: 0.440–0.622), respectively (Table 4 and Fig. 3A). After adjusting for previous OHE, the AUROCs for plasma NfL and GFAP levels distinguishing patients with MHE remained at 0.727

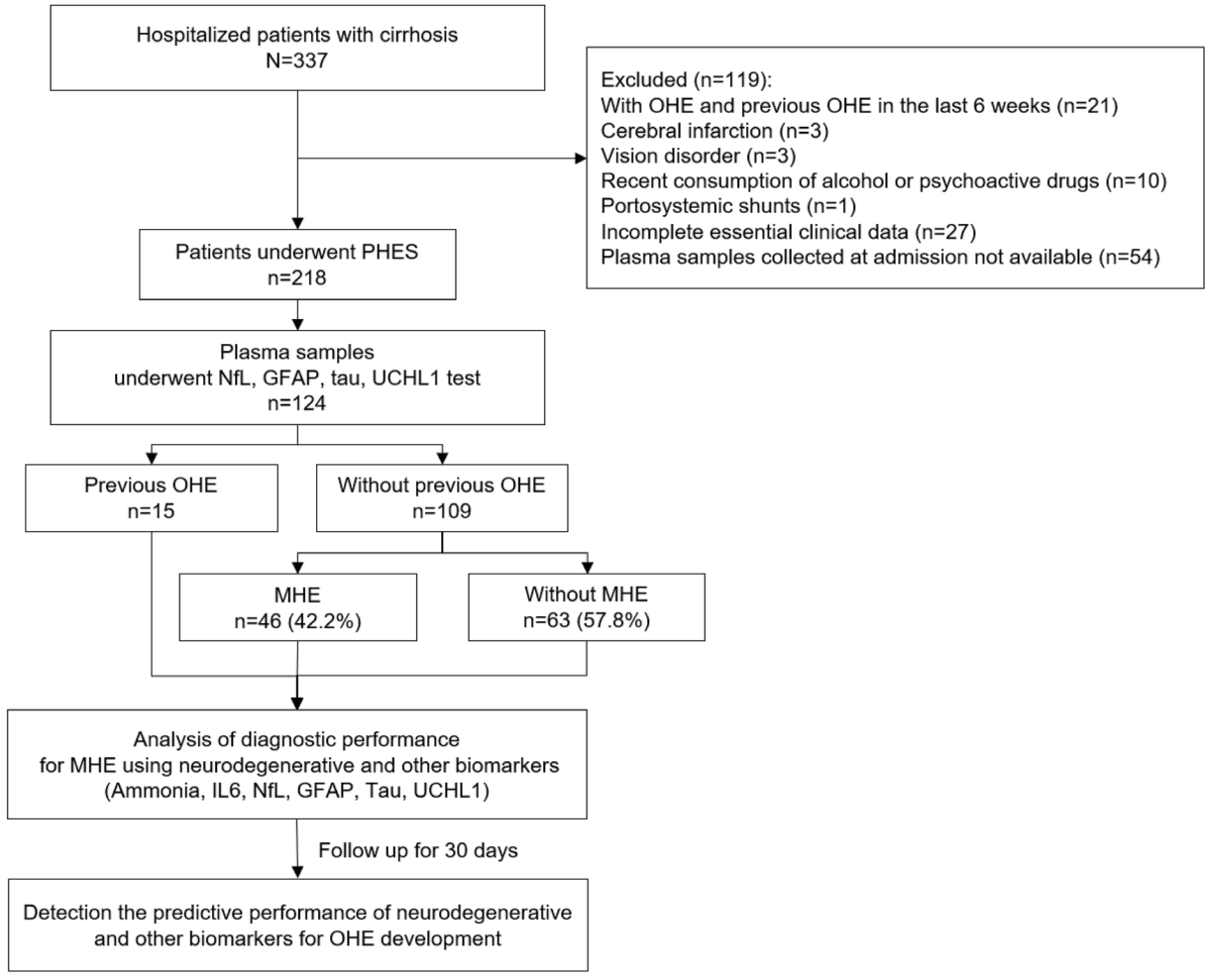

Fig. 1. Flowchart of the study. MHE, minimal hepatic encephalopathy; OHE, overt hepatic encephalopathy; NfL, neurofilament light chain; GFAP, glial fibrillary acidic protein; UCHL1, ubiquitin carboxy-terminal hydrolase L1; IL-6, interleukin-6; PHES, psychometric hepatic encephalopathy score.

(95% CI: 0.633–0.808) and 0.704 (95% CI: 0.609–0.788), respectively (Fig. 3B). However, the AUROCs for tau and UCHL1 levels distinguishing patients with MHE were 0.536 (95% CI: 0.416–0.653) and 0.540 (95% CI: 0.421–0.657), respectively (Table 4 and Fig. 3A, B).

Specifically, for NfL levels, a threshold of 25.7 pg/mL showed a sensitivity, specificity, accuracy, PPV, and NPV of 80.4%, 57.1%, 67.0%, 57.8%, and 80.0%, respectively (Table 4). For GFAP levels, a threshold of 116.4 pg/mL showed sensitivity, specificity, accuracy, PPV, and NPV of 80.4%, 60.3%, 68.8%, 59.7%, and 80.9%, respectively (Table 4).

Diagnostic performance of the combination of plasma neurodegenerative biomarkers

After analyzing different combinations of these biomarkers, we found that a combination of NfL, GFAP, tau, and UCHL1 was capable of diagnosing MHE with an AUROC of 0.748 (95% CI: 0.662–0.821) and accuracy, sensitivity, specificity, PPV, and NPV of 71.0%, 71.9%, 71.6%, 68.3%, and 75.0%, respectively, in all patients (Table 4 and Fig. 3C), and an AUROC of 0.764 (95% CI: 0.673–0.840) and accuracy, sensitivity, specificity, PPV, and NPV of 72.5%, 71.7%, 73.0%, 66.0%, and 78.0%, respectively, in those without previous OHE (Table 4 and Fig. 3D).

Risk factors for 30-day OHE development

OHE developed in 18 (14.5%) patients during the 30-day follow-up period. Patients with previous OHE showed a higher OHE development rate than those without previous OHE (5/15, 33.3% *vs*. 13/109, 11.9%, HR = 3.265, 95% CI: 1.163–9.164, χ^2 = 4.405, p = 0.025, Table 5). Patients who developed OHE during follow-up had higher IL-6 levels (34 pg/mL [IQR 22; 510] *vs*. 17 pg/mL [IQR 9; 33], p = 0.003) and MELD scores (27 [IQR 22; 29] *vs*. 15 [IQR 9; 20], p < 0.001) than those without OHE. In the univariate Cox regression model, GFAP (HR = 1.002, 95% CI: 1.000–1.003, χ^2 = 1.283, p = 0.065), IL-6 (HR = 1.002, 95% CI: 1.001–1.003, χ^2 = 4.095, p < 0.001), and MELD score (HR = 1.121, 95% CI: 1.075–1.170, χ^2 = 4.458, p < 0.001) were also associated with OHE development (Table 5). However, NfL (HR = 1.001, 95% CI: 0.998–1.004, χ^2 =0.359, p = 0.549), tau (HR = 0.989, 95% CI: 0.948–1.031, χ^2 = 0.373, p = 0.596), and UCHL1 (HR = 1.000, 95% CI: 1.000–1.001, χ^2 = 0.759, p = 0.548) were not associated with OHE development (Table 5).

Moreover, multivariate Cox regression analysis showed that GFAP (HR = 1.003, 95% CI: 1.000–1.005; χ2 = 0.105, p = 0.044), IL-6 (HR = 1.003, 95% CI: 1.001–1.004; χ2 = 4.626, p < 0.001), and MELD score (HR = 1.139, 95% CI:

Table 1. Baseline demographic and clinical characteristics of patients with hepatic cirrhosis in relation to minimal hepatic encephalopathy (n = 124)

	Total (n = 124)	With MHE (n = 57)	Without MHE (n = 67)	*p*
Sex				0.120
Female	29 (23.4)	17 (29.8)	12 (17.9)	
Male	95 (76.6)	40 (70.2)	55 (82.1)	
Age, y (IQR)	54 (47–62)	56 (51–66)	50 (41–57)	<0.001*
Etiology of hepatic cirrhosis				
Virus-related (HBV/HCV/combined)	94 (75.8)	44 (77.2)	50 (74.6)	0.739
Chronic alcohol-related	4 (3.2)	1 (1.8)	3 (4.8)	0.624
Autoimmune	4 (3.2)	2 (4.3)	2 (3.1)	1.000
Cholestatic	2 (1.6)	2 (4.3)	0 (0)	0.209
Schistosomiasis	3 (2.4)	3 (5.3)	0 (0)	0.094
Others (drug-induced/ metabolic/ unknown)	17 (13.7)	5 (8.8)	12 (17.9)	0.192
Plasma neurodegenerative biomarkers				
NfL, ρg/mL	28.1 (19.3–48.2)	34.2 (26.9–78.8)	22.4 (16.5–35.5)	<0.001*
GFAP, ρg/mL	144.0 (80.2–203.4)	173.0 (126.2–239.4)	97.6 (68.2–178.8)	<0.001*
tau, ρg/mL	10.4 (6.4–17.9)	8.4 (5.6–17.2)	11.6 (8.1–19.2)	0.048*
UCHL1, ρg/mL	48.7 (34.9–91.6)	49.1 (32.9–81.6)	48.2 (37.3–98.1)	0.546
Clinical factors				
Ammonia, μmol/L	65 (49–86)	75 (53–96)	57 (47–76)	0.029*
IL-6, ρg /mL	20 (9–36)	23 (13–35)	18 (9–36)	0.589
Albumin, g/L	32 (28–35)	31 (28–35)	32 (29–36)	0.315
PCT, ng/mL	0.30 (0.10–0.50)	0.30 (0.14–0.61)	0.28 (0.15–0.45)	0.696
CRP, mg/L	8.8 (3.8–15.7)	9 (4.0–17.5)	8.8 (3.8–15.0)	0.794
MELD	16 (9–21)	14 (9–23)	16 (10–20)	0.971
MELD-Na	18 (13–25)	18 (11–26)	18 (14–24)	0.777
Previous OHE	15 (12)	11 (19)	4 (6)	0.024*
Events				
Ascites	98 (79.0)	44 (77.1)	54 (80.5)	0.644
Pleural effusion	55 (44.3	26 (45.6)	29 (43.2)	0.728
Portal hypertension	76 (61.2)	32 (56.1)	44 (65.6)	0.334
Esophageal and gastric varices	60 (48.3)	25 (48.3)	35 (52.2)	0.403
Gastrointestinal hemorrhage	8 (6.4)	2 (3.5)	6 (8.9)	0.220
SBP	42 (38.8)	23 (40.3)	19 (28.3)	0.161
Pulmonary infection	31 (25.0)	18 (31.5)	13 (19.4)	0.120
Fungal infection	4 (3.2)	0 (0)	4 (5.9)	0.062
Urinary system infection	3 (2.4)	3 (5.2)	0 (0)	0.058
Other infections (*e.g.*, CMV, EBV)	29 (23.3)	13 (22.8)	16 (23.8)	0.889

Data are expressed as the mean ± standard deviation, median (interquartile range), or number (percentage), as appropriate. * $p < 0.05$, comparison between patients with MHE and those without MHE. NfL, GFAP, tau, and UCHL1 were measured in 124 patients; ammonia was measured in 120 patients; IL-6 was measured in 91 patients; albumin was measured in 121 patients; PCT and CRP were measured in 100 patients. MHE, minimal hepatic encephalopathy; OHE, overt hepatic encephalopathy; NfL, neurofilament light chain; GFAP, glial fibrillary acidic protein; UCHL1, ubiquitin carboxy-terminal hydrolase L1; IL-6, interleukin-6; PCT, procalcitonin; CRP, C-reactive protein; MELD, model for end-stage liver disease; IQR, interquartile range; SBP, spontaneous bacterial peritonitis; CMV, cytomegalovirus; EBV, Epstein-Barr virus.

Table 2. Associations of plasma neurodegenerative biomarkers with demographic and clinical characteristics in hospitalized patients with hepatic cirrhosis without previous overt hepatic encephalopathy

	NfL	GFAP	tau	UCHL1
Demographics				
Age	0.390***	0.393***	−0.165	−0.019
Female sex	0.072	−0.232*	−0.108	−0.128
Hepatic virus related	0.045	−0.050	−0.081	−0.070
MHE	0.388***	0.305***	−0.145	−0.050
Clinical factors				
Ammonia	−0.167	0.041	−0.053	−0.113
IL-6	0.308**	0.284*	0.174	0.146
Albumin	−0.195*	−0.131	−0.148	−0.219*
PCT	0.100	−0.017	0.026	0.198
CRP	0.186	0.171	0.184	0.182
MELD	−0.035	−0.052	0.236*	0.053
MELD-Na	0.025	−0.097	0.332**	0.089
Days of hospitalization	0.140	−0.018	0.276**	0.249**
Events				
Ascites	0.110	−0.026	0.109	0.282**
Pleural effusion	0.134	0.110	0.221*	0.095
Portal hypertension	0.067	−0.170	0.029	0.052
Esophageal and gastric varices	−0.013	−0.196*	0.022	−0.106
Gastrointestinal hemorrhage	0.001	−0.156	0.014	−0.044
SBP	0.143	0.027	0.149	0.087
Pulmonary infection	0.175	0.174	0.024	0.162

Data in the table are Spearman's correlation coefficients. * $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$. MHE, minimal hepatic encephalopathy; NfL, neurofilament light chain; GFAP, glial fibrillary acidic protein; UCHL1, ubiquitin carboxy-terminal hydrolase L1; IL-6, interleukin-6; PCT, procalcitonin; CRP, C-reactive protein; MELD, model for end-stage liver disease; MELD-Na, MELD-sodium; SBP, spontaneous bacterial peritonitis.

1.072–1.210; $\chi 2 = 5.821$, $p < 0.001$) were significantly associated with OHE development (Table 5).

Discussion

The present prospective study systematically explored neurodegenerative biomarkers—NfL, GFAP, tau, and UCHL1—as well as traditional markers, including IL-6 and ammonia, and disease severity (MELD/MELD-Na scores) in the diagnosis of MHE and risk factors for predicting OHE development in patients with hepatic cirrhosis. MHE was diagnosed in 46.0% of patients with hepatic cirrhosis. There were significantly higher plasma levels of NfL and GFAP, and lower tau levels in patients with MHE compared to those without MHE. In addition, plasma NfL and GFAP levels were positively correlated with MHE development in patients without previous OHE. Furthermore, plasma NfL and serum ammonia levels were independently associated with MHE. A combination of NfL, GFAP, tau, and UCHL1 was capable of diagnosing MHE in all cirrhotic patients with an accuracy, sensitivity, and specificity of 71.0%, 71.9%, and 71.6%, respectively, and in those without previous OHE with an accuracy, sensitivity, and specificity of 72.5%, 71.7%, and 73.0%, respectively. Furthermore, GFAP, IL-6, and MELD scores were identified as risk factors for subsequent 30-day OHE development in patients with hepatic cirrhosis.

Previous studies have demonstrated that astrocytic swelling, dysfunction of astrocytes and neurons, mitochondrial impairment, and severe damage or demise of neuronal cells have been identified as contributors to the development of HE,[3,4,17] indicating the potential application of plasma neurodegenerative biomarkers, such as NfL, GFAP, tau, and UCHL1, for diagnosing MHE and predicting OHE development. NfL, an intra-axonal structural protein, is reported to be a marker of astrocyte swelling, astrocytic activation, synaptic connection, lymphatic activation, and blood-brain barrier integrity, and can be used to detect subtle central nervous system (CNS) injury.[31] GFAP is an astrocytic cytoskeletal protein that is upregulated during astrocytic activation[32,33] and is a sensitive biomarker for detecting and tracking reactive astrogliosis.[34] The astrocyte terminal foot containing GFAP is an important part of the blood-brain barrier, synaptic connection, and lymphatic system,[35–38] mediating relevant damage in HE.[39] Tau is a microtubule-related protein in neurons, whose dysfunction triggers pathological events such as impaired axonal transport, alterations in synapses, and mitochondrial function.[40] UCHL1, a neuron-specific protein, is an important component of the ubiquitin-proteasome pathway, which is engaged in removing abnormal proteins and protecting the neuron from potentially toxic proteins.[41] Loss of UCHL1 contributes to central and peripheral neurodegeneration and brain injury.[28] Indeed, increased serum levels of NfL and GFAP have been reported to be independently associated

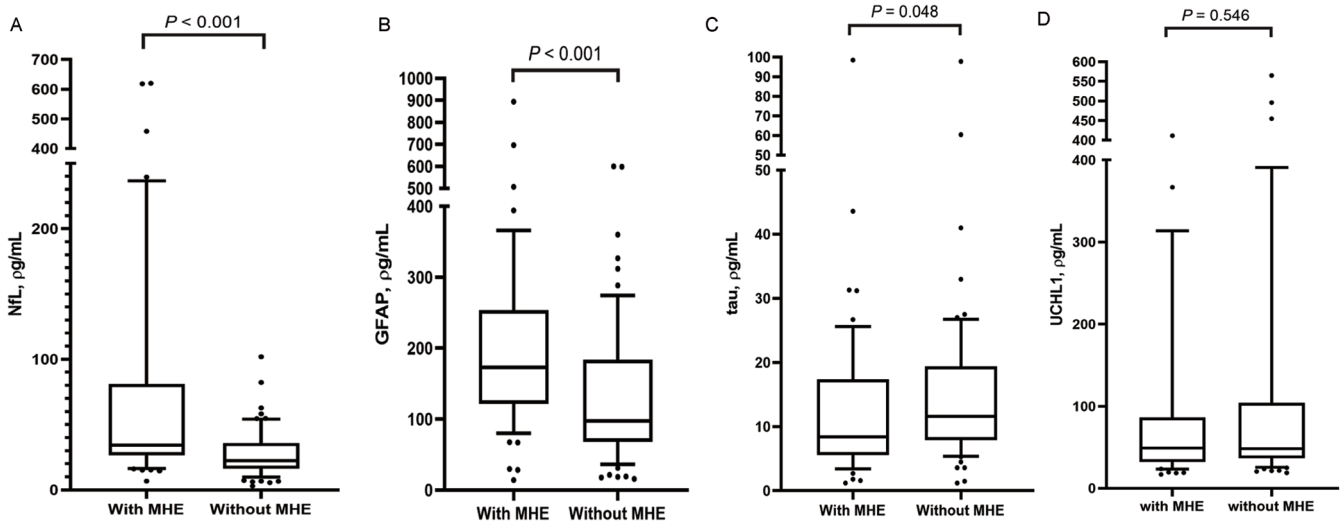

Fig. 2. Plasma neurodegenerative biomarker levels in cirrhotic patients with and without MHE. (A) Plasma NfL levels in cirrhotic patients with and without MHE. (B) Plasma GFAP levels in cirrhotic patients with and without MHE. (C) Plasma tau levels in cirrhotic patients with and without MHE. D. Plasma UCHL1 levels in cirrhotic patients with and without MHE. Data are presented as boxplots with median, IQR, and range. The differences in neurodegenerative biomarker levels between cirrhotic patients with MHE and those without MHE were evaluated using the Mann–Whitney U test. MHE, minimal hepatic encephalopathy; NfL, neurofilament light chain; GFAP, glial fibrillary acidic protein; UCHL1, ubiquitin carboxy-terminal hydrolase L1; IQR, interquartile range.

with MHE in cirrhotic patients in Western countries.[25,26] This study is the first time to explore the ability of NfL, GFAP, tau, and UCHL1, both individually and in combination to diagnose MHE and predict OHE development in Chinese patients with hepatic cirrhosis. The median MELD score in this cohort was 16 (IQR 9–21), which significantly differs from the median MELD score of 11 (IQR 9–16) or 13 (IQR 9–18) reported in European patients with hepatic cirrhosis.[25,26] Another novel point of the present study is that we compared the performance of these biomarkers, alone and in combination, in the same cohort, and found that NfL and GFAP showed similar performance in screening MHE, with a combination of NfL, GFAP, tau, and UCHL1 being capable of diagnosing MHE.

Table 3. Associations of baseline plasma neurodegenerative biomarkers and other potential variables with minimal hepatic encephalopathy as identified in the multivariable logistic regression model

Variable	OR (95% CI)	*p*
Total cohort (n = 124)		
Age	1.058 (1.013–1.105)	0.011*
Previous OHE	3.237 (0.785–13.346)	0.104
NfL	1.027 (1.006–1.048)	0.013*
GFAP	0.999 (0.994–1.003)	0.557
tau	1.000 (0.970–1.031)	0.992
Ammonia	1.021 (1.006–1.036)	0.007*
Without previous OHE (n=109)		
Age	1.069 (1.018–1.122)	0.008*
NfL	1.024 (1.003–1.045)	0.024*
GFAP	1.000 (0.994–1.005)	0.927
tau	1.003 (0.973–1.034)	0.848
Ammonia	1.018 (1.002–1.034)	0.025*

*$p < 0.05$. OHE, overt hepatic encephalopathy; NfL, neurofilament light chain; GFAP, glial fibrillary acidic protein; UCHL1, ubiquitin carboxy-terminal hydrolase L1; OR, odds ratio; CI, confidence interval.

The main findings of the present study are consistent with those observed in previous experimental studies.[18,29,30,42,43] The pathological substrate of permanent disability in various neurological disorders has not been reported for MHE; however, neuronal injury, neurite degeneration, and synaptic alterations have previously been observed in rat HE models.[18,29] NfL is a cytoplasmic protein involved in neuroaxonal injury,[30] and cerebrospinal fluid (CSF) and serum or plasma NfL levels have been shown to be associated with brain injury.[42,43] In the present study, plasma NfL levels were significantly associated with MHE (Table 3) in multivariate logistic analysis, indicating that there may be potential neuroaxonal injury in cirrhotic patients with MHE, accompanied by significantly increased plasma NfL levels.

In the present study, MELD score, IL-6, and GFAP were identified as risk factors for 30-day OHE development. This finding partly supports previous studies indicating that elevated IL-6 levels and MELD scores can identify cirrhotic patients at higher risk for OHE.[44] Recently, de Wit *et al*. reported that NfL, but not GFAP, was a potential biomarker for OHE in cirrhotic patients.[45] However, the predictive capabilities of NfL, GFAP, tau, and UCHL1 for OHE have not been simultaneously explored in Chinese patients with hepatic cirrhosis. In the present study, we found that MELD score, IL-6, and GFAP—but not NfL, tau, or UCHL1—were independent predictors of OHE development, as identified in multivariate Cox regression analysis. This finding further suggests that MELD score and IL-6 are closely associated with subsequent OHE attacks. Notably, the HR for GFAP in predicting OHE was low, with the lower limit of the 95% CI at 1.000, raising concerns about its clinical significance. The relatively small sample size in the present study may explain the low HR. It is recognized that the pathogenesis of HE in both acute and chronic liver failure involves astrocyte swelling, cerebral oxidative stress, microglial activation, and altered neurotransmission,[4] and the primary risk factors contributing to the development and acceleration of OHE in complex clinical scenarios remain to be elucidated. Therefore, data from larger, multicenter studies encompassing a range of disease severities are required.

Table 4. Performance of plasma neurodegenerative biomarkers, along with serum ammonia and interleukin-6 for diagnosing minimal hepatic encephalopathy

Test	AUROC (95% CI)	Cutoff	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	*p*-value
Total patients (n = 124)								
Ammonia	0.616 (0.523–0.704)	66	65.0	60.4	68.7	60.4	68.7	0.030
IL-6	0.536 (0.428–0.641)	14.4	50.5	73.1	41.5	33.3	79.4	0.596
NfL	0.719 (0.632–0.796)	26.7	66.9	75.4	59.7	61.4	74.1	<0.001
GFAP	0.690 (0.600–0.770)	104.1	66.9	80.7	55.2	60.5	77.1	<0.001
tau	0.603 (0.511–0.689)	8.8	64.5	54.4	73.1	63.3	65.3	0.050
UCHL1	0.532 (0.440–0.622)	38	58.1	38.6	74.6	56.4	58.8	0.546
NfL & GFAP	0.732 (0.645–0.807)	0.3324	66.9	89.5	47.8	59.3	84.2	<0.001
NfL & GFAP & tau	0.744 (0.657–0.818)	0.4113	71.0	73.7	70.2	67.7	75.8	<0.001
NfL & GFAP & tau & UCHL1	0.748 (0.662–0.821)	0.4207	71.0	71.9	71.6	68.3	75.0	<0.001
NfL & GFAP & ammonia	0.763 (0.677–0.836)	0.3916	67.7	69.8	71.6	66.1	75.0	<0.001
NfL & GFAP & tau & UCHL1 & ammonia	0.773 (0.688–0.854)	0.3691	67.7	71.7	70.2	65.5	75.8	<0.001
Without previous OHE (n = 109)								
Ammonia	0.586 (0.486–0.681)	66	62.9	54.8	68.3	53.5	69.4	0.149
IL6	0.595 (0.490–0.693)	14.4	53.2	89.5	39.7	26.6	93.9	0.133
NfL	0.727 (0.633–0.808)	25.7	67.0	80.4	57.1	57.8	80.0	<0.001
GFAP	0.704 (0.609–0.788)	116.4	68.8	80.4	60.3	59.7	80.9	<0.001
tau	0.536 (0.416–0.653)	5.6	65.1	30.4	86.3	50.0	73.3	0.653
UCHL1	0.540 (0.421–0.657)	27.6	45.0	95.7	23.5	36.1	92.3	0.558
NfL & GFAP	0.755 (0.663–0.832)	0.3324	67.9	91.3	50.8	57.5	88.9	0.045
NfL & GFAP & tau	0.759 (0.668–0.836)	0.4113	72.5	73.9	73.0	66.7	79.3	<0.001
NfL & GFAP & tau & UCHL1	0.764 (0.673–0.840)	0.4184	72.5	71.7	73.0	66.0	78.0	<0.001
NfL & GFAP & ammonia	0.751 (0.651–0.830)	0.3916	68.8	66.7	74.6	63.6	77.0	<0.001
NfL & GFAP & tau & UCHL1 & ammonia	0.762 (0.668–0.839)	0.3691	67.0	66.7	73.0	62.2	76.7	<0.001

p-value indicates the significance of the AUROC of each biomarker. MHE, minimal hepatic encephalopathy; OHE, overt hepatic encephalopathy; NfL, neurofilament light chain; GFAP, glial fibrillary acidic protein; UCHL1, ubiquitin carboxy-terminal hydrolase L1; IL-6, interleukin-6; AUROC, area under the receiver operating characteristic curve; PPV, positive predictive value; NPV, negative predictive value.

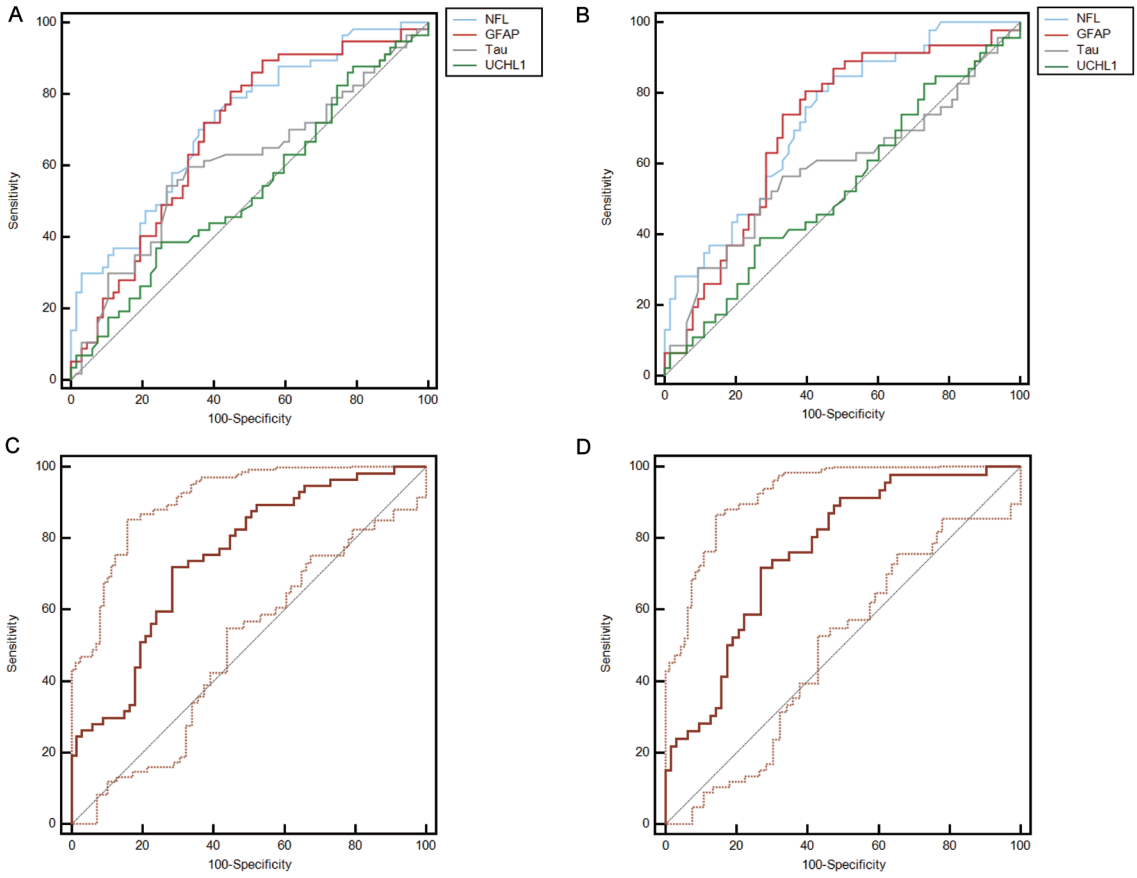

Fig. 3. Receiver operating characteristic curve of plasma neurodegenerative biomarker levels in patients with hepatic cirrhosis. (A) All eligible patients (n = 124); (B) Patients without previous OHE (n = 109). (C) Receiver operating characteristic curve of the combination of NfL, GFAP, and UCHL in all eligible patients (n = 124); (D) Receiver operating characteristic curve of the combination of NfL, GFAP, and UCHL1 in patients without previous OHE (n = 109). MHE, minimal hepatic encephalopathy; OHE, overt hepatic encephalopathy; NfL, neurofilament light chain; GFAP, glial fibrillary acidic protein; UCHL1, ubiquitin carboxy-terminal hydrolase L1.

Additionally, we further investigated the associations of the four neurodegenerative biomarkers with the causes of cirrhosis but failed to find any associations with hepatitis virus-related cirrhosis. This suggests that the diagnostic utility of NfL and GFAP is not specifically confined to patients with hepatitis virus-related cirrhosis. This finding aligns with previous observations that ongoing harmful alcohol consumption did not significantly impact serum GFAP levels in patients with alcoholic cirrhosis.[18] Interestingly, there was an inverse correlation between the levels of plasma NfL and UCHL1 and serum albumin levels in cirrhotic patients in the present study (Table 2). This finding partially supports previous observations, in which UCHL1 showed inverse correlations with albumin levels.[46] Additionally, the present study demonstrated that tau and UCHL1 were associated with delayed hospitalization, but only tau was associated with the MELD score. Except for tau with pleural effusion, UCHL1 with ascites, and GFAP with esophageal and gastric varices, no correlations were found between these four neurodegenerative biomarkers and PCT, CRP, hepatitis virus-related cirrhosis, or complications associated with hepatic cirrhosis. A significant association has been reported between serum UCHL1 concentrations and albumin quotients [Q(A) = albumin (CSF)/albumin (serum)], which reflects blood-brain barrier disruption following traumatic brain injury.[47] However, no significant relationship was observed between plasma UCHL1 levels and MHE or OHE. Thus, the clinical detection of pathophysiological roles of axonal transport and blood-brain barrier impairments in the development of MHE or OHE warrants further investigation in future studies.

The present study has several limitations. Firstly, the number of patients included in the study was relatively small due to the research budget, which could only support the detection of a limited number of samples using SiMoA technology, and the study was conducted in a single research center with Chinese patients. Therefore, a larger, multicenter study that includes cirrhotic patients from diverse ethnic backgrounds is needed to validate the diagnostic value of plasma NfL and GFAP levels for MHE and the predictive value of plasma GFAP levels for OHE. Secondly, there were no healthy controls in the study, and the absence of blood biomarker concentrations in healthy controls prevented us from assessing the age dependence of plasma NfL and GFAP levels. Thirdly, we did not track longitudinal changes in plasma neurodegenerative biomarker levels, particularly in patients without MHE at baseline who developed MHE during the follow-up period and in patients with MHE at baseline who recovered during the follow-up period. We plan to address this gap with a longitudinal study to investigate the role of neural alterations in MHE development, recovery, and relapse. Fourthly, we lacked CSF biomarker levels, which could have provided crucial insights into the association between cerebral conditions and peripheral alterations. Fifthly, due to the high cost, not every patient underwent a brain MRI to rule out CNS diseases, and thus CNS diseases were primarily ruled out through a history of brain diseases,

Table 5. Risk factors for the development of overt hepatic encephalopathy in patients with hepatic cirrhosis in univariate and multivariate Cox regression model analyses (n = 124)

	Univariate Cox regression analysis		Multivariate Cox regression analysis	
	HR (95% CI)	***p***	**HR (95% CI)**	***p***
Age	1.024 (0.986–1.064)	0.216		
Female sex	0.461 (0.179–1.189)	0.109		
Neurodegenerative biomarkers				
NfL	1.000 (0.998–1.003)	0.863		
GFAP	1.002 (1.000–1.003)	0.065*	1.003 (1.000–1.005)	0.044*
tau	0.989 (0.948–1.031)	0.596		
UCHL1	1.000 (1.000–1.001)	0.548		
Clinical factors				
Ammonia	1.002 (0.987–1.018)	0.765		
IL-6	1.002 (1.001–1.003)	<0.001*	1.003 (1.001–1.004)	<0.001*
Creatinine	1.003 (0.998–1.008)	0.275		
Albumin	1.002 (0.915–1.097)	0.969		
MELD	1.121 (1.075–1.170)	<0.001*	1.139 (1.072–1.210)	<0.001*
MELD-Na	1.038 (0.971–1.110)	0.277		
Previous OHE	3.265 (1.163–9.164)	0.025*	2.168 (0.459–10.238)	0.329
Events				
Ascites	1.088 (0.307–3.858)	0.896		
Pleural effusion	1.237 (0.467–3.274)	0.669		
Portal hypertension	0.973 (0.372–2.546)	0.955		
Esophageal and gastric varices	0.705 (0.245–2.026)	0.516		
Gastrointestinal hemorrhage	0.048 (0.000–4.40e8)	0.795		
SBP	1.319 (0.459–3.790)	0.607		
Pulmonary infection	1.386 (0.502–3.821)	0.529		
Fungal infection	0.931 (0.201–4.320)	0.927		
Urinary system infection	0.047 (0.000–5.05e6)	0.746		
Other infections (*e,g.*, CMV, EBV)	1.189 (0.368–3.844)	0.773		
HE treatment	34.268 (0.456–2573)	0.109		

*p-value < 0.1. HR, hazard ratio; CI, confidence interval; NfL, neurofilament light chain; GFAP, glial fibrillary acidic protein; UCHL1, ubiquitin carboxy-terminal hydrolase L1; IL-6, interleukin-6; MELD, model for end-stage liver disease; MELD-Na, MELD-sodium; SBP, spontaneous bacterial peritonitis; CMV, cytomegalovirus; EBV, Epstein-Barr virus.

previous MRI reports, and physical examination. In the future, we will cooperate with the imaging department or apply for relevant funding support to consider this important issue.

Conclusions

The combination of plasma levels of NfL, GFAP, tau, and UCHL1 exhibits competent performance in diagnosing MHE, and MELD score, IL-6, and GFAP, rather than plasma NfL, tau, and UCHL1 levels, appear to be the predictors of 30-day OHE development in patients with hepatic cirrhosis.

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Conflict of interest

QN has been an Editorial Board Member of *Journal of Clinical and Translational Hepatology* since 2024. The other authors have no conflict of interests related to this publication.

Author contributions

Study design (QC, TC, QN), research performance, data acquisition (QC, YL, XZ, TC, QN), data analysis (QC, TC, QN), paper writing and revision (QC, TC, QN), analysis tool sup-

port, and other assistance (ZY, TL, MZ, YN, WL, LH, YF, XL). All authors approved the final version of the manuscript and the authorship list.

Ethical statement

The study protocol was approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (S196), accordance with the Declaration of Helsinki (as revised in 2013). Informed consent was obtained from all patients.

Data sharing statement

The datasets used and/or analyzed during the present study are available from the corresponding authors upon reasonable request.

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