Evaluation of IL-6 for Stepwise Diagnosis of Minimal Hepatic Encephalopathy in Patients With Liver Cirrhosis

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Diagnosis of minimal hepatic encephalopathy (MHE) requires psychometric testing, which is time-consuming and often neglected in clinical practice. Elevated Interleukin-6 (IL-6) serum levels have been linked to MHE. The aim of this study was to investigate the usefulness of IL-6 as a biomarker in a stepwise diagnostic algorithm to detect MHE in patients with liver cirrhosis. A total of 197 prospectively recruited patients without clinical signs of hepatic encephalopathy (HE) served as the development cohort. Another independent cohort consisting of 52 patients served for validation purposes. Psychometric Hepatic Encephalopathy Score (PHES) was applied for the diagnosis of MHE. Fifty (25.4%) patients of the development cohort presented with MHE. Median IL-6 levels were more than twice as high in patients with MHE than in patients without HE (16 vs. 7 pg/mL; $P < 0.001$). On multivariable logistic regression analysis, higher IL-6 levels (odds ratio 1.036; 95% confidence interval [CI] 1.009-1.064; $P = 0.008$) remained independently associated with the presence of MHE. IL-6 levels ≥ 8pg/mL discriminated best between patients with and without MHE in receiver operating characteristic (ROC) analysis (area under the ROC 0.751). With a cutoff value of ≥7 pg/mL, further elaborate testing with PHES could be avoided in 38% of all patients with a sensitivity of 90% (95% CI 77%-96%) and a negative predictive value (NPV) of 93% (95% CI 84%-98%). This diagnostic accuracy was confirmed in the validation cohort (sensitivity 94%; NPV 93%). *Conclusion:* Using IL-6 serum levels as a biomarker in a stepwise diagnostic algorithm to detect MHE could substantially reduce the number of patients requiring testing with PHES and in turn the workload. IL-6 may have especially helped in patients who are unable to perform other screening tests. (*Hepatology Communications* 2022;6:1113-1122).

Hepatic encephalopathy (HE) represents one of the major complications of liver cirrhosis and is associated with poor prognosis.[1] Its clinical presentation ranges from subclinical neurocognitive impairments to life-threatening coma.[2] Minimal HE (MHE) is defined as the earliest and mildest stage of HE with a prevalence of 20%-80%.[3] By definition, MHE is clinically inapparent and only detectable by specialized tests.[4]

Currently, the Psychometric Hepatic Encephalopathy Score (PHES) represents the gold standard for the detection of MHE.[5,6] Although the PHES has been available for more than 20 years and has been validated in many countries, testing for MHE is often neglected

Abbreviations: AUROC, area under the receiver operating characteristic; CCM, Cirrhosis Center Mainz; CI, confidence interval; CNS, central nervous system; CRP, C-reactive protein; HE, hepatic encephalopathy; IL, interleukin; IQR, interquartile range; MELD, Model for End-Stage Liver Disease; MHE, minimal hepatic encephalopathy; NPV, negative predictive value; NRI, net reclassification improvement; OHE, overt hepatic encephalopathy; PHES, Psychometric Hepatic Encephalopathy Score; PPV, positive predictive value; ROC, receiver operating characteristic; WBC, white blood cell count.

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in routine clinical practice.[7] One important reason in clinical practice is the time-intensive nature of the test. Given the negative impact of MHE on quality of life, ability to drive and prognosis, there is an urgent need for new cost-effective and time-saving diagnostic tools.[8-10]

In recent years, new testing strategies like the Stroop EncephalApp to simplify testing for MHE have been developed.[11] However, even this app-based test takes about 10 to 15 minutes, and every patient has to conduct the entire test. Therefore, stepwise diagnostic algorithms including an easily applicable screening test to reduce the number of patients who have to be tested with the more time-consuming specialized tests could be an important step in increasing the test frequency in routine clinical practice.

Despite intensive research, the pathogenesis of HE is still poorly understood.[12] However, systemic inflammation appears to negatively influence hyperammonemia-associated neurotoxicity.[13] The pleiotropic cytokine interleukin-6 (IL-6) plays an important role in systemic inflammation, exerts multiple effects in the central nervous system (CNS), and is elevated in various pathological conditions.[14] IL-6 contributes to the disruption of the blood–brain barrier by increasing the permeability of CNS-derived endothelial cells, which in turn leads to an elevated ammonia influx into the CNS.[15]

In line with these preclinical findings, measurement of IL-6 serum levels as a marker of systemic inflammation allows us to identify patients with liver cirrhosis at increased risk for developing an episode of overt hepatic encephalopathy (OHE).[16] Additionally, higher levels of markers of inflammation like IL-6 were found in patients with MHE compared to patients without HE.[17,18] Montoliu et al. could show in their study that every patient with MHE had IL-6 serum levels of above 11 pg/mL, and ultimately proposed this cutoff. However, their study lacked both a sufficient sample size as well as a validation.[17] Taken together, IL-6 may represent a suitable candidate biomarker for screening for MHE, especially in patients who are unable to perform other established screening tests. Therefore, the aims of this study were (1) to evaluate the accuracy of IL-6 to predict the presence of MHE in patients with liver cirrhosis; and (2) to determine and validate cutoff values to use IL-6 as a primary screening tool in a stepwise diagnostic algorithm to detect MHE.

Patients and Methods

DEVELOPMENT COHORT

In total, 270 patients with liver cirrhosis prospectively enrolled in a database between March 2017 and August 2020 at the Cirrhosis Center Mainz (CCM) of the University Medical Center of the Johannes Gutenberg University in Mainz, Germany, were screened for eligibility for this study. Detailed characteristics of this study were published elsewhere.[16,19]

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The leading etiology of underlying liver disease was determined according to clinical, serological, and histological findings. Diagnosis of liver cirrhosis was established by histology or a combination of conclusive appearance in ultrasound, radiological imaging, endoscopic features of portal hypertension, and medical history. Blood biochemistry was assessed in all patients. Venous ammonia was obtained from each patient without tourniquet in a semifrozen tube, and samples were transported cooled and processed within 10 minutes.

Patients were excluded if they fulfilled one or more of the following criteria: evidence of an active infection, previous episode of OHE, chronic alcohol consumption during the last 3 months, any intake of psychotropic drugs or opioids, the presence of preterminal comorbidities (heart disease [New York Heart Association III-IV], chronic obstructive pulmonary disease [Gold C and D], renal failure with creatinine >1.5 mg/dL), the presence of hepatocellular carcinoma or other malignancies, transjugular intrahepatic portosystemic shunt, neurological comorbidities (i.e., dementia or history of stroke), history of recent head trauma, and severe electrolyte disorders (serum sodium <130 mg/dL or >150 mg/dL). Reasons for elective hospitalization were either to perform liver biopsy, paracentesis (without evidence of spontaneous bacterial peritonitis), esophagogastroduodenoscopy with expected band ligation, or evaluation for liver transplantation.

As described elsewhere, patients showing increased serum levels of IL-6, C-reactive protein (CRP) or white blood cell count (WBC) were carefully examined regarding potential infections using physical examination and detailed anamnesis.[16] If clinically indicated, additional investigations were initiated to exclude active infections. To further exclude confounding factors, patients with inflammatory diseases like rheumatoid arthritis were excluded from this study.

VALIDATION COHORT

For validation purposes, a *post-hoc* analysis of data of 52 independent patients recruited prospectively during elective hepatic venous pressure gradient measurements at the CCM were analyzed. Inclusion and exclusion criteria for these patients were the same as described previously. According to a sample-size calculation based on the development data, 46 patients would be needed for the validation cohort (significance level of $\alpha = 5\%$ and a power of 80%).

DIAGNOSIS OF HE

First, every patient was examined by an experienced hepatologist to rule out OHE. The presence of HE grade 1 was diagnosed after detailed neurological examination according to the West Haven criteria.[2] Patients with HE grade 1 were excluded from further analysis in this study.

Testing for MHE was done using the portosystemic encephalopathy syndrome test, which produces the PHES. Interpretation of PHES was done as previously described with German norms.[20] PHES was never performed on the same day of any other intervention to exclude potential confounding factors. All tests were performed in a quiet, lighted room between 9:00 AM and 4.00 PM. A score <−4 was considered as pathological.[10]

DETERMINATION OF IL-6 SERUM LEVELS

IL-6 serum levels were determined at the day of study inclusion immediately after testing with PHES using a commercially available chemiluminescence immunoassay (Cobas e 411 Analyzer; F. Hoffmann-La Roche AG, Basel, Switzerland). Patients did not have to be fasting, and blood sampling was performed in the morning hours between 8 AM and 12 PM.

FOLLOW-UP EVALUATION

Patients of the development cohort were followed up during routine visits every 6 months in the outpatient clinic of the CCM, as described elsewhere.[21] At each visit or during unplanned hospitalizations, each patient was examined by an experienced hepatologist to rule in or rule out OHE. The presence of OHE was diagnosed after detailed neurological examination according to the West Haven criteria.

ETHICS

The study was conducted in accordance with the ethical guidelines of the 1975 Declaration of

Helsinki (6th revision, 2008). The studies for both cohorts were approved by the ethics committee of the Landesärztekammer Rheinland–Pfalz. Written informed consent was obtained from all participants.

STATISTICAL ANALYSIS

Quantitative data are expressed as medians with interquartile ranges (IQRs), and pairwise comparisons for quantitative variables were performed with an unpaired Student *t* test or with the Mann-Whitney U test. Categorical variables are expressed as frequencies and percentages. For comparison of two or more patient groups, a chi-square test was applied.

To identify predictors for the presence of MHE, a multivariable analysis using a logistic regression model was conducted. Here, only significant variables ($P < 0.05$) on univariable analysis were included into the model. Additionally, we determined the net reclassification improvement (NRI) and integrated discrimination improvement (INI) to compare the increase of the predictive value of a model containing IL-6, Model for End-Stage Liver Disease (MELD), and albumin levels compared with a model containing only IL-6.

The discriminative ability of IL-6 for the identification of patients with MHE was analyzed with help of the area under the curve of receiver operating characteristic (AUROC) curves and its respective 95% CI. Thresholds for IL-6 were determined based on two different optimality criteria. First, we determined the ideal cutoff value maximizing the Youden's index. In order to use IL-6 as a first screening test in a stepwise diagnostic algorithm, we next identified the first cutoff with at least 90% sensitivity. Additionally, we identified a second (higher) cutoff with at least 90% specificity.

For all tests, we used a 0.05 level to define statistically relevant deviations from the respective null hypotheses.

All data were analyzed using IBM SPSS Statistics Version 23.0, GraphPad Prism Version 8.4.1, and SAS Version 9.4.

Results

COHORT DESCRIPTION

A total of 270 patients with liver cirrhosis were prospectively recruited. Of these, 73 were excluded from further analysis (Fig. 1). The baseline characteristics of the remaining 197 patients are displayed in Table 1. These patients served as the development cohort. At the day of study inclusion, 50 (25.4%) patients were classified as having MHE based on an abnormal PHES. Median age of the development cohort was 61 years (IQR 54, 67) and most patients were male (n = 110, 55.8%). Median IL-6 serum level in the total cohort was 8 pg/mL (IQR 5, 18).

The validation cohort consisted of 52 patients with liver cirrhosis without a history of OHE. Baseline characteristics of this cohort are displayed in Supporting Table S1. In this cohort, 16 (30.8%) patients were classified as having MHE. Median IL-6 serum levels were 15 pg/mL (IQR 6, 28) at study inclusion.

COMPARISON OF BASELINE CHARACTERISTICS BETWEEN PATIENTS WITH AND WITHOUT MHE IN THE DEVELOPMENT COHORT

Median IL-6 serum levels were more than twice as high in patients with MHE compared to patients without HE (16 vs. 7 pg/mL; $P < 0.001$) (Fig. 2A). IL-6 serum levels did not differ significantly between patients with PHES values from −5 to −10 and from −11 to −16 (Fig. 2B). The same holds true for the comparison of IL-6 serum levels in the groups of patients with a PHES of 0 to −4 and 5 to 0. There was a negative correlation between IL-6 serum levels and PHES with a spearman's rho of −0.392 ($P < 0.001$) (Fig. 2C).

Patients with MHE were in a more severe stage of their disease, as reflected by a higher MELD score and a higher frequency of a history of ascites. Moreover, CRP serum levels as well as albumin levels differed significantly between both groups, while there was no difference in WBC or sodium. Detailed comparisons between both cohorts are displayed in Table 1.

VARIABLES ASSOCIATED WITH THE PRESENCE OF MHE IN THE DEVELOPMENT COHORT

To identify variables associated with the presence of MHE in patients of the development cohort, a

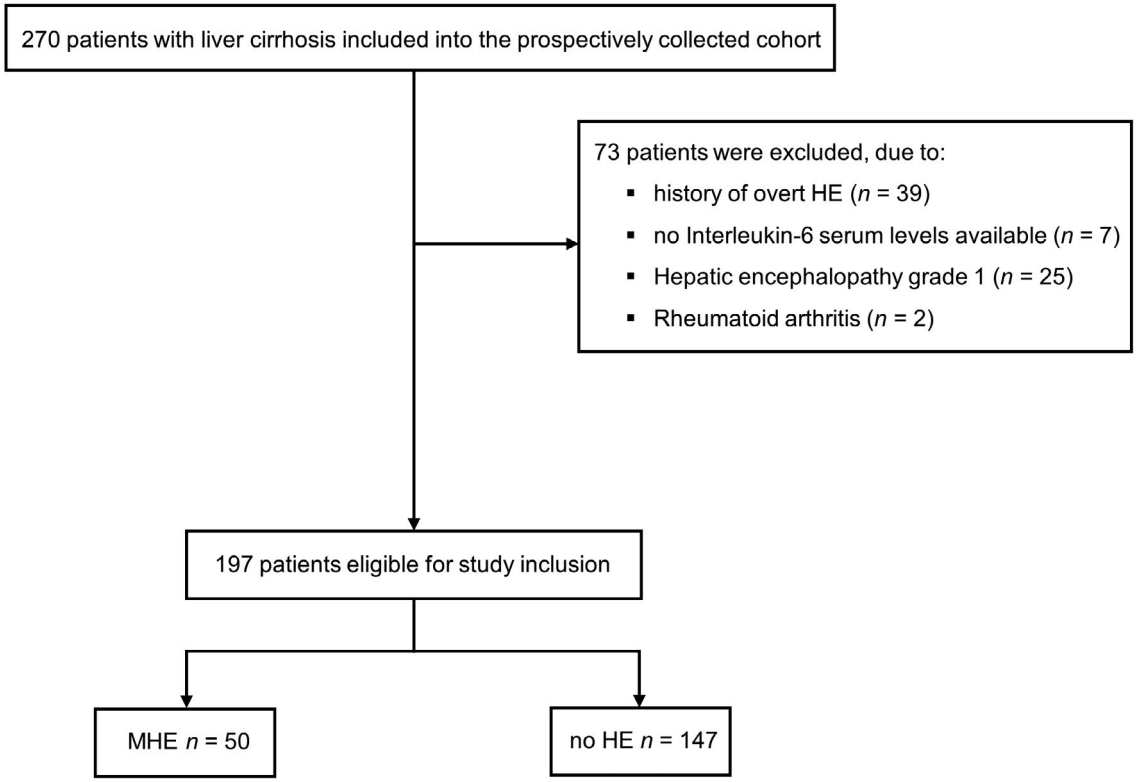

FIG. 1. Flow diagram showing the reasons for dropout of patients.

multivariable logistic regression model was conducted. Variables with a $P < 0.05$ in univariable analyses (Table 1; MHE vs. no HE) were subsequently included in the multivariable model. Here, IL-6 remained significantly associated with the presence of MHE with an odds ratio of 1.036 (95% CI 1.009-1.064; $P = 0.008$). CRP, albumin levels, MELD score, and history of ascites were not independently associated with the presence of MHE (Table 2).

To examine the increase of the predictive value of a model containing IL-6, MELD, and albumin levels compared with a model only containing IL-6, we determined the NRI and IDI. The benefit of adding MELD and albumin to IL-6 was small with an NRI of 0.04762 and an IDI of −0.0001249.

DIAGNOSTIC ACCURACY OF IL-6 IN DETECTING MHE IN THE DEVELOPMENT COHORT

To test the diagnostic performance of IL-6 in detecting MHE, ROC curve analyses were performed (Fig. 3). The AUROC curve for IL-6 was 0.751 (95% CI 0.675-0.827; $P < 0.001$) in the development cohort (Table 3). In comparison, the AUROC for CRP levels was 0.608 (95% CI 0.508-0.709; $P = 0.023$), the AUROC for WBC was 0.541 (95% CI 0.446-0.635; $P = 0.387$), the AUROC for MELD score was 0.622 (95% CI 0.534-0.710), the AUROC for Child-Pugh score was 0.663 (95% CI 0.571-0.755), the AUROC for history of ascites was 0.609 (95% CI 0.0.519-0.699), and the AUROC for albumin was 0.596 (95% CI 0.491-0.702). In patients with low MELD scores (MELD < 15), the diagnostic performance of IL-6 to detect MHE remained stable with an AUROC of 0.736 (95% CI 0.648-0.824). The optimal cutoff value for IL-6 to identify MHE as determined by the Youden's index was ≥8 pg/mL in the development cohort. This cutoff yielded a sensitivity and specificity of 82% and 57%, respectively (Table 3).

Additionally, we identified a cutoff value with at least 90% sensitivity for the potential use of IL-6 as a primary screening tool in a stepwise diagnostic algorithm, to rule out patients without MHE and avoid

TABLE 1. DEMOGRAPHICS AND CLINICAL CHARACTERISTICS OF THE DEVELOPMENT COHORT, PATIENTS WITH MHE, AND PATIENTS WITHOUT HE AT THE TIME OF STUDY INCLUSION

Variable		All Patients (n = 197)	Patients With MHE (n = 50)	Patients Without HE (n = 147)	*P* Value
Age, years (IQR)		61 (54, 67)	62 (54, 71)	60 (54, 66)	0.268
Male gender, n (%)		110 (55.8)	32 (64.0)	78 (53.1)	0.178
Etiology	Alcohol, n (%)	60 (30.5)	22 (44.0)	38 (25.9)	0.036
	Viral hepatitis, n (%)	43 (21.8)	9 (18.0)	34 (23.1)	
	NAFLD, n (%)	24 (12.2)	5 (10.0)	19 (12.9)	
	Cholestatic/ autoimmune, n (%)	29 (14.7)	2 (4.0)	27 (18.4)	
	Other/mixed, n (%)	41 (20.8)	12 (24.0)	29 (19.7)	
Median MELD score (IQR)		10 (7, 13)	12 (8, 15)	9 (7, 13)	0.01
Child-Pugh A/B/C, n (%)		131/56/10 (66.5/28.4/5.1)	22/21/7 (44.0/42.0/14.0)	109/35/3 (74.1/23.8/2.0)	<0.001
History of ascites, n (%)		94 (47.7)	32 (64.0)	62 (42.2)	0.008
Sodium, mmol/L (IQR)		138 (137; 140)	138 (135; 140)	139 (137; 140)	0.06
Albumin, g/L (IQR)		35 (30, 39)	32 (26, 39)	35 (31, 39)	0.041
WBC, /nL (IQR)		5.5 (4.2, 7.4)	5.6 (4.4, 7.9)	5.5 (4.1, 7.2)	0.387
CRP, mg/L (IQR)		4 (2, 8)	6 (3, 19)	3 (2, 7)	0.023
IL-6, pg/mL (IQR)		8 (5, 18)	16 (9, 42)	7 (4, 12)	<0.001
Ammonia, µmol/L (IQR)*		45 (35, 55)	46 (33, 54)	44 (36, 56)	0.823
MHE, n (%)		50 (25.4%)	50 (100%)	0 (0%)	

Note: Data are expressed as medians and IQRs or as frequencies and percentages.
Abbreviation: NAFLD, nonalcoholic fatty liver disease.
*Measured in 182 patients.

further elaborate testing with specialized tools like PHES. A cutoff value of ≥7 pg/mL resulted in a sensitivity of 90% with a negative predictive value (NPV) of 93% (Table 3). Using this cutoff, additional testing could have been avoided in 75 (38%) patients of the development cohort (Fig. 4). Of these, 5 (7%) had MHE and would have been classified as false negative.

Sensitivity, specificity, positive predictive value (PPV), and NPV for a cutoff with at least 90% specificity (≥25 pg/mL) are displayed in Table 3.

VALIDATION OF IL-6 AS A PREDICTOR FOR MHE IN AN INDEPENDENT COHORT

To ascertain the validity of IL-6 as a predictor for MHE, the discriminative ability of IL-6 was evaluated in an independent cohort of 52 patients with liver cirrhosis. Characteristics of this cohort are displayed in Supporting Table S1.

In the validation cohort, the diagnostic performance of IL-6 could be confirmed with an AUROC of 0.722 (95% CI 0.573-0.872, $P = 0.011$) (Table 4; Supporting Fig. S1). A cutoff value of ≥8 pg/mL yielded a sensitivity of 88% and a specificity of 39%. Applying the lower cutoff value of ≥7 pg/mL, sensitivity and NPV remained high with 94% and 93%, respectively (Table 4). Using this cutoff, additional testing could have been avoided in 15 (29%) patients of the validation cohort. Of these, only 1 (7%) patient had a MHE.

ABILITY OF THE PROPOSED STEPWISE DIAGNOSTIC ALGORITHM INCLUDING IL-6 AND PHES TO PREDICT THE DEVELOPMENT OF OHE

Median follow-up time was 491 (IQR 328, 726) days, and 12 (6.1%) patients were lost to follow-up. In total, 26 patients developed an episode of OHE after a median of 118 (IQR 62, 425) days. Of these first-time OHE episodes, 19 patients developed HE grade 2 according to the West Haven criteria, 5 patients developed HE grade 3, and 2 patients developed HE grade 4. Cumulative OHE incidences for the subgroups defined by the proposed algorithm in Fig. 4 are shown in Supporting Fig. S2. The group of patients with IL-6 ≥ 7 pg/mL and PHES < –4

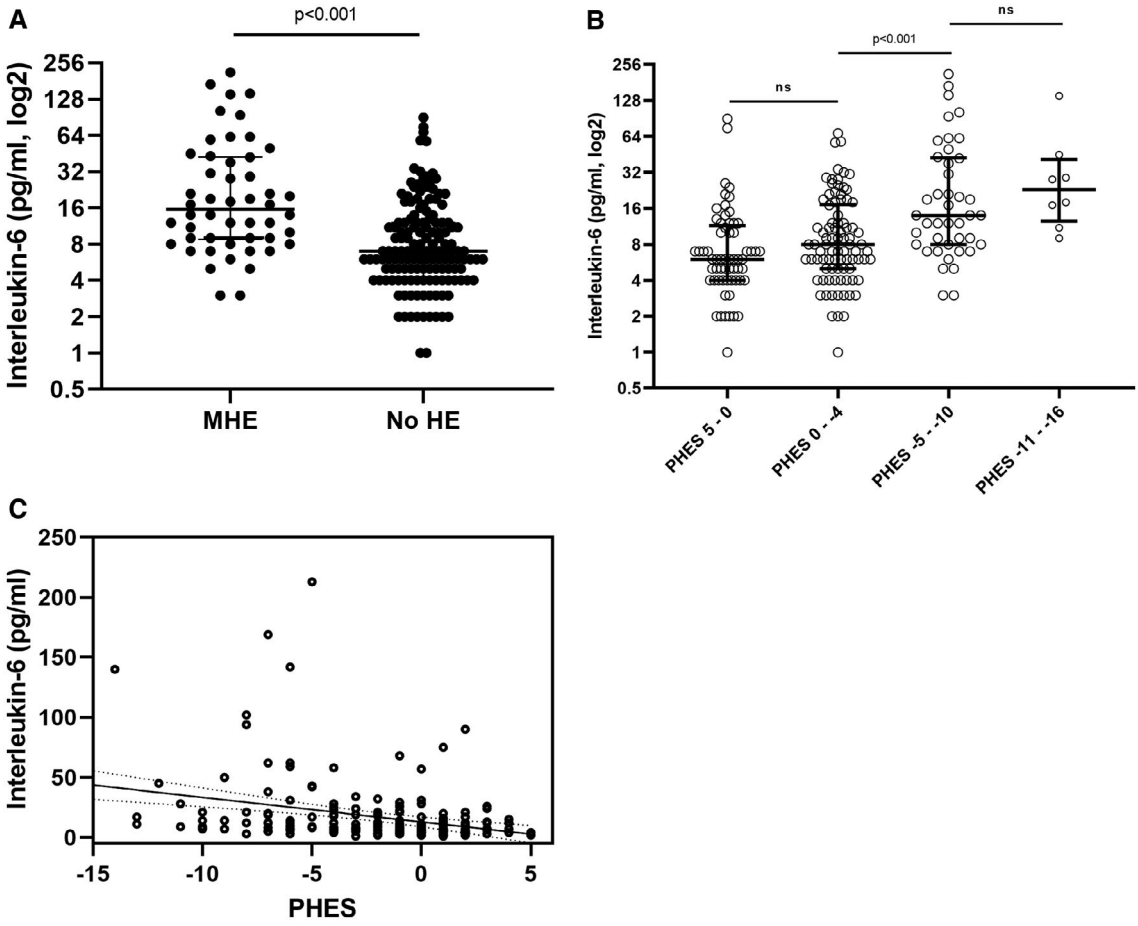

FIG. 2. Median serum levels of IL-6 in patients with and without MHE and across groups with different performances in PHES. (A) IL-6 serum levels in patients with liver cirrhosis with (n = 50) or without MHE (n = 147). (B) IL-6 serum levels across four groups with different performances in PHES. (C) Correlation between serum levels of IL-6 and PHES (Spearman's rho = −0.392, $P < 0.001$). Abbreviation: ns, not significant.

was at the highest risk for the development of OHE during follow-up ($P = 0.007$).

Within the first year of follow-up, 16 patients developed an episode of OHE (8.6%). Development of OHE within the first year was most frequent in the group of patients with IL-6 ≥ 7 pg/mL and PHES < −4 (22.2%), followed by patients with IL-6 ≥ 7 pg/mL and PHES ≥ −4 (9.2%). There was only one OHE episode in patients with IL-6 < 7 pg/mL, regardless of results in PHES (Fig. 4).

TABLE 2. LOGISTIC REGRESSION ANALYSES OF VARIABLES ASSOCIATED WITH THE PRESENCE OF MHE

Development Cohort	Odds Ratio (95% CI)	*P* Value
IL-6	1.036 (1.009-1.064)	0.008
MELD	0.976 (0.877-1.087)	0.663
Albumin	1.010 (0.935-1.090)	0.802
CRP	1.014 (0.977-1.051)	0.462
History of ascites	0.708 (0.323-1.553)	0.389

Note: Not significant were a history of OHE and CRP in the total cohort, and CRP in the cohort without patients with a history of OHE.

Discussion

Systemic inflammation is a key driver in the pathogenesis of HE. In the study presented here, we found that IL-6 is independently associated with the presence of MHE in patients with liver cirrhosis; however, the diagnostic performance of IL-6 as a stand-alone

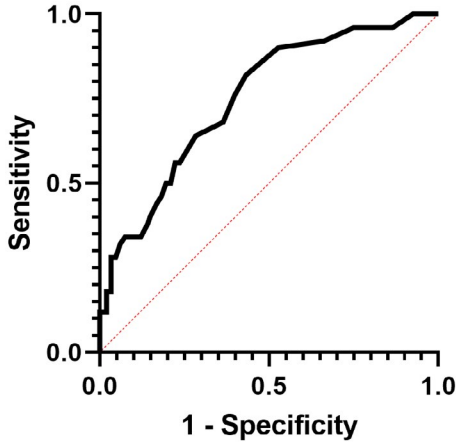

FIG. 3. Discriminative ability of IL-6 to detect MHE in the development cohort of patients with liver cirrhosis (AUC = 0.751; 95% CI 0.675-0.827; $P < 0.001$).

TABLE 3. PERFORMANCE OF DIFFERENT CUTOFFS OF IL-6 TO PREDICT THE PRESENCE OF MHE IN THE DEVELOPMENT COHORT

	Youden's Index: ≥8 pg/mL	Lower Cutoff: ≥7 pg/mL	Higher Cutoff: ≥25 pg/mL
AUROC	0.751 (0.675-0.827)		
Sensitivity	82% (68-91)	90% (77-96)	34% (22-49)
Specificity	57% (49-65)	48% (39-56)	91% (84-95)
PPV	39% (30-50)	37% (28-46)	55% (36-72)
NPV	90% (82-95)	93% (84-98)	80% (73-86)

Note: 95% confidence intervals given in brackets.

parameter to detect MHE was only mediocre. We could demonstrate that IL-6 is a suitable candidate biomarker as an alternative primary screening tool in a stepwise diagnostic algorithm for the detection of MHE. Using an IL-6 cutoff of ≥7 pg/mL, elaborate testing with PHES could have been avoided in about one-third of patients with liver cirrhosis, leading to a meaningful reduction of workload, thereby eventually increasing the proportion of patients screened routinely for MHE. Additionally, we found that adding IL-6 to psychometric testing with PHES has the ability to improve risk stratification of patients regarding the development of OHE.

Blood-based biomarkers for the screening for MHE could be of substantial benefit to increase the frequency of testing for MHE in routine clinical practice. Although we found a strong association between higher IL-6 serum levels and the presence of MHE, which replicates prior smaller studies,[17,18] the diagnostic performance of IL-6 as a stand-alone parameter to detect MHE and the correlation between IL-6 and PHES turned out to be only mediocre. Besides the fact that MHE can, by definition, only be diagnosed by specialized neurophysiological assessment to identify the presence of cognitive deficits, our findings suggest that IL-6, as a stand-alone test, would have no sufficient accuracy to reliable identify patients with MHE. However, even the ideal cutoff as determined by the Youden's index in our cohorts had a remarkably high sensitivity of 82%, whereas a lower cutoff of ≥7 pg/mL even reached a sensitivity of 90%. This indirectly indicates that low-grade inflammation appears to be a prerequisite for the development and presence of MHE.

Several studies, including ours, demonstrated a positive correlation between IL-6 serum levels and the presence of MHE in patients with liver cirrhosis.[15,16] The high sensitivity and NPV of even low IL-6 serum levels in our study suggest that some level (albeit low) of systemic inflammation must be present for MHE to develop, supporting previous findings by Shawcross et al.[13] This hypothesis is supported by the lack of significant differences in IL-6 serum levels between patients with excellent results in PHES (5 to 0 points) and patients with slight but non-pathologic impairment (0 to −4 points), while there was a highly significant increase in serum IL-6 levels in patients with MHE and PHES results of −5 to −10 points compared to the group of patients with 0 to 4 points.

MHE is by definition a phenotype and cannot be diagnosed by a laboratory parameter such as IL-6. Additionally, we are aware that IL-6 may not be the screening tool of first choice in every patient with liver cirrhosis in a stepwise diagnostic algorithm to detect MHE in clinical practice. Here, one of the most important reasons may be cost, while other suitable screening tools, like the Animal Naming Test, are free of charge. However, given its independency of education, gender or examiner, IL-6 may represent a more appropriate tool for initial screening. Another advantage of implementing IL-6 into routine HE workup is its additional predictive value for the development of an OHE episode, which is even independent of underlying MHE/covert HE.[16] In our current study, the addition of IL-6 to PHES appeared to improve

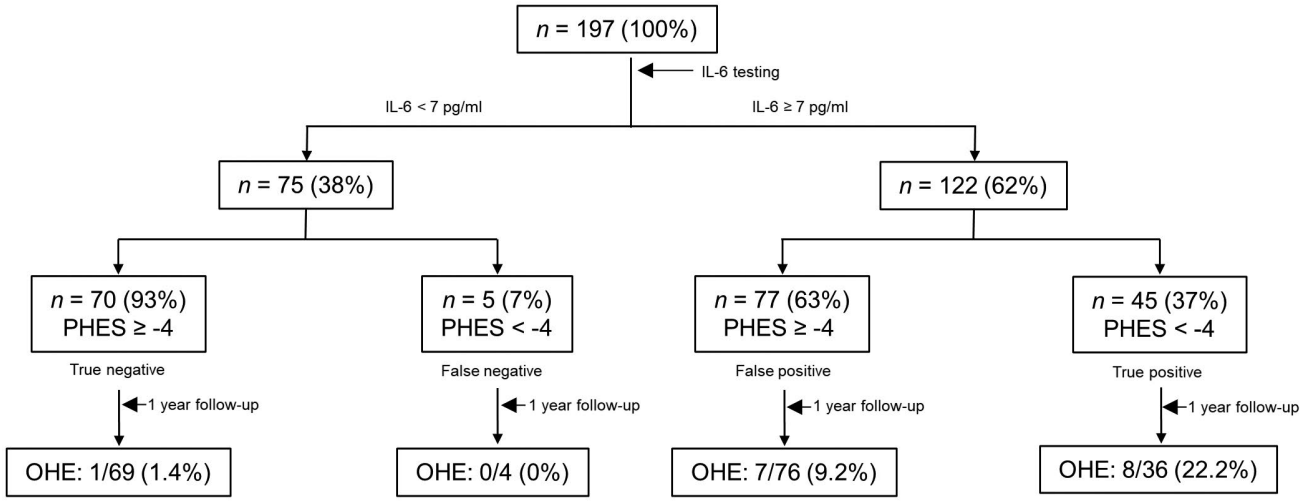

FIG. 4. Flow diagram showing the proposed stepwise diagnostic algorithm using IL-6 (cutoff ≥ 7 pg/mL) as a prescreening tool followed by PHES for the detection of MHE in the development cohort. Patients were followed for 1 year regarding development of OHE. Twelve patients were lost to follow-up. A total of 16 episodes of OHE occurred during follow-up.

TABLE 4. PERFORMANCE OF DIFFERENT CUTOFFS OF IL-6 TO PREDICT THE PRESENCE OF MHE IN THE VALIDATION COHORT

	Youden's Index of Development Cohort: ≥8 pg/mL	Lower Cutoff: ≥7 pg/mL	Higher Cutoff: ≥25 pg/mL
AUROC		0.722 (0.573-0.872)	
Sensitivity	88% (60-98)	94% (68-100)	38% (16-64)
Specificity	39% (24-56)	39% (24-56)	78% (60-89)
PPV	39% (24-56)	41% (25-58)	43% (18-70)
NPV	88% (60-98)	93% (66-100)	74% (57-86)

Note: In brackets: 95% confidence interval.

the predictive ability regarding the development of OHE and may therefore facilitate the decision to initiate primary prophylaxis. In this context, IL-6 may even have merit in monitoring treatment success. Randomized trials indicated that IL-6 levels decrease under treatment with lactulose or probiotics.[22,23] However, this needs to be further investigated in future studies.

Our study has some limitations that have to be acknowledged. For instance, the cross-sectional and observational nature of our study does not allow valid conclusions, neither on pathomechanisms nor on causality of the association observed between higher IL-6 serum levels and the presence of MHE. Moreover, we did not investigate the longitudinal variation in IL-6 serum levels and are therefore unable to assess whether day-to-day changes in IL-6, gender, or age may affect the prognostic usefulness. Our validation cohort is comparatively small. However, it was large enough to validate the discriminative ability of IL-6 and our proposed cutoffs. Nevertheless, external validation in larger cohorts has to be conducted in future studies. A limitation of the applicability of IL-6 is that patients with highly elevated levels need to be thoroughly examined for possible infections. Finally, patients with preterminal comorbidities, chronic inflammatory conditions, or severe electrolyte disorders were not included into this study, thus not allowing for a uniform use of IL-6 in all clinical encountered constellations. However, parts of these limitations appear only secondary, considering that screening for MHE in patients with preterminal comorbidities may be clinically not meaningful.

In conclusion, IL-6 may be helpful in selecting patients for subsequent psychometric testing and may reduce the need for psychometric testing in one-third of cases. In addition, IL-6 serum levels ≥ 7 pg/mL alone might prompt initiation of anti-HE treatment in patients unable to undergo psychometric testing. In the future, a larger longitudinal validation cohort is needed that would also allow assessment of IL-6 response to treatment effect.

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