Original Article

Protein-S-100-beta is increased in patients with decompensated cirrhosis admitted to ICU

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A R T I C L E   I N F O

A B S T R A C T

Background: Hepatic encephalopathy (HE) is highly prevalent in patients with liver diseases. The pathophysiology of HE is centered on the synergic role of hyperammonemia and systemic inflammation. However, some data suggest altered functioning of the blood–brain barrier (BBB). Assessing BBB function is challenging in clinical practice and at the bedside. Protein-S-100 Beta (PS100-Beta) could be a useful peripheral marker of BBB permeability in HE. This study aimed to assess plasmatic PS100-Beta levels in a prospective cohort of patients admitted to the intensive care unit (ICU) with decompensated cirrhosis with and without overt HE.

Methods: We retrospectively evaluated a prospective cohort of cirrhotic patients admitted to the ICU from October 2013 to September 2015 that had an available plasmatic PS100-Beta measurement. Patients with previous neurological impairment or limitation of intensive or resuscitative measures were excluded. Overt HE was defined as West-Haven grades 2 to 4. The patients were compared to a control cohort of outpatient clinic cirrhotic and non-cirrhotic patients explored for isolated elevation of liver enzymes. After ICU discharge, the patients were followed for at least 3 months for the occurrence of overt HE. Adverse outcomes (liver transplantation or death) were collected. The ability of PS100-Beta – in combination with other factors – to predict overt HE was evaluated in a multivariate analysis using logistic regression. Likelihood ratios were used to determine the effects and calculate odds ratios (OR). Survival analysis was performed by using the Kaplan–Meier method and survival between groups was compared using a Log-rank test.

Results: A total of 194 ICU patients and 207 outpatients were included in the study. Increased levels of plasmatic PS100-Beta were detected in the ICU decompensated cirrhotic patients compared with the outpatients ([0.15±0.01] mg/L *vs.* [0.08±0] mg/L, *P* <0.001). ICU patients with overt HE had higher levels of PS100-Beta ([0.19±0.03] mg/L) compared with the ICU patients without overt HE ([0.13±0.01] mg/L) (*P*=0.003). PS100-Beta levels did not differ in outpatients with F 0–3 compared to F 4 fibrosis (*P*=0.670). PS100-Beta values were correlated with Child-Pugh score (*P* <0.001), Model for End-Stage Liver Disease (MELD) score (*P*=0.004), C-

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reactive protein (*P* <0.001), ammonemia (*P* <0.001), and chronic liver failure consortium (CLIF-C) organ failure (*P* <0.001) and CLIF-C acute-on-chronic (*P*=0.038) scores, but not with leukocytes (*P*=0.053), procalcitonin (PCT) (*P*=0.107), or the lymphocyte-to-neutrophil ratio in ICU patients (*P*=0.522). In a multivariate model including age, ammonemia, PS100-Beta, PCT, MELD, presence of transjugular portosystemic shunt, and sodium level, the diagnostic performance was 0.765 for the diagnosis of overt HE. Patients with a PS100-Beta level <0.12 mg/L had a better overall survival (*P*=0.019) and a better survival without liver transplantation (*P*=0.013).

Conclusions: Serum levels of PS100-Beta are elevated in ICU patients with decompensated cirrhosis, and even more so in those displaying overt HE, and the levels are correlated with outcome. This suggests an increase in the permeability of the BBB in these patients.

Introduction

Hepatic encephalopathy (HE) corresponds to all the neurological or neuropsychological symptoms caused by a liver disease and/or a portosystemic shunt.[1] Symptoms can range from a mild neurocognitive impairment without abnormalities on neurological examination and only detected by neuropsychological testing, called minimal HE, to asterixis, altered consciousness, and coma, which is easily detected by abnormal neurological examination and is termed overt HE.[1–3] One-third of patients present with overt HE at diagnosis and two-thirds in their disease history. Overt HE is frequently encountered in intensive care unit (ICU) patients with decompensated cirrhosis.

The pathophysiology of HE is still debated, although the synergic role of hyperammonemia and systemic inflammation is now largely accepted.[4–7] The former abnormalities could be a direct consequence of the dysbiosis observed in cirrhotic patients.[8] However, other abnormalities have been described. Our group recently showed, both in animal models and in humans by different techniques, that the blood–brain barrier (BBB) that constitutes a unique interface between the brain and different organs, especially the liver, has an impaired functioning in cirrhotic patients and even more if they display overt HE.[9–12] We also showed that HE patients accumulate several xenobiotics in their cerebrospinal fluid, which is probably directly due to the increased permeability of the BBB.[9]

Assessing BBB function is challenging in clinical practice and at the bedside. Protein-S-100 Beta (PS100-Beta) is a small dimeric cytosolic protein synthesized in astrocytes and Schwann cells. Several lines of evidence have suggested that PS100-Beta could constitute a useful peripheral marker of BBB permeability.[13–17] High serum levels of PS100-Beta (normal values <0.10 μg/L) have been reported in different neurological diseases and could be correlated to brain lesions and altered BBB permeability; these diseases include subarachnoid hemorrhage,[18,19] stroke,[20] cardiac arrest,[21] traumatic brain injury,[22] seizures/status epilepticus,[13,23] or cerebral tumors.[17]

Here, we evaluated plasmatic PS100-Beta levels in a prospective cohort of patients with decompensated cirrhosis admitted to our hepatological ICU and compared those to a cohort of outpatient clinic cirrhotic and non-cirrhotic patients evaluated for isolated elevated liver enzymes.

Methods

This study was performed in the Department of Hepatogastroenterology at La Pitié-Salpêtrière Hospital, Sorbonne University, which is a tertiary care center in Paris, France. Samples were collected after obtaining written informed consent, in accordance with the local ethics committee of La Pitié-Salpêtrière Hospital (institutional committee), Paris, France (Comité de Protection des Personnes 33_13, April 24, 2013).

ICU decompensated cirrhotic patients

All patients admitted to our hepatological ICU from October 2013 to September 2015 and included in our prospective cohort were retrospectively evaluated for inclusion in the study (Figure 1). Inclusion criteria were the presence of a decompensated cirrhosis, admission to ICU, and having an available plasmatic measurement of PS100-Beta. Exclusion criteria were the presence of a previous neurological impairment or limitation of intensive/resuscitative measures. If several ICU admissions were available for the same patient, only the first one was considered. Cirrhosis was diagnosed based on clinical, biological, and/or anatomopathological features. Demographic data (age, gender) and natural history of cirrhosis (etiology, presence of a transjugular intrahepatic portosystemic shunt [TIPS], hepatocellular carcinoma) were collected at admission. The severity of cirrhosis was evaluated by the Child-Pugh and the Model for End-Stage Liver Disease (MELD) scores. Reasons for admission to ICU were retrieved and the presence or absence of variceal bleeding, spontaneous bacterial peritonitis, HE (see below), acute alcoholic hepatitis, infection, and shock were noted. Severity at ICU admission was assessed using chronic liver failure consortium (CLIF-C) organ failure (CLIF-C OF) and CLIF-C acute-on-chronic liver failure (ACLF, CLIF-C ACLF) scores and the Glasgow coma scale (GCS).

All patients underwent a neurological evaluation by a senior hepatologist within the first 24 h after admission using the West-Haven (WH) score. Neurological advice was looked for when needed to exclude a differential diagnosis. Patients were considered to have overt HE when WH scores were 2 to 4; patients with WH grades 0 to 1 were classed as having no overt HE.

Outpatient clinic patients

A prospective cohort of our outpatient clinic patients who were examined for isolated elevated liver enzymes were evaluated. All underwent a FibroTest (F) as a non-invasive marker of fibrosis.[24] Patients with previous neurological impairment or neurological or psychiatric diseases were excluded. The following data were collected: demographic data (age, gender) and etiology of elevated liver enzymes.
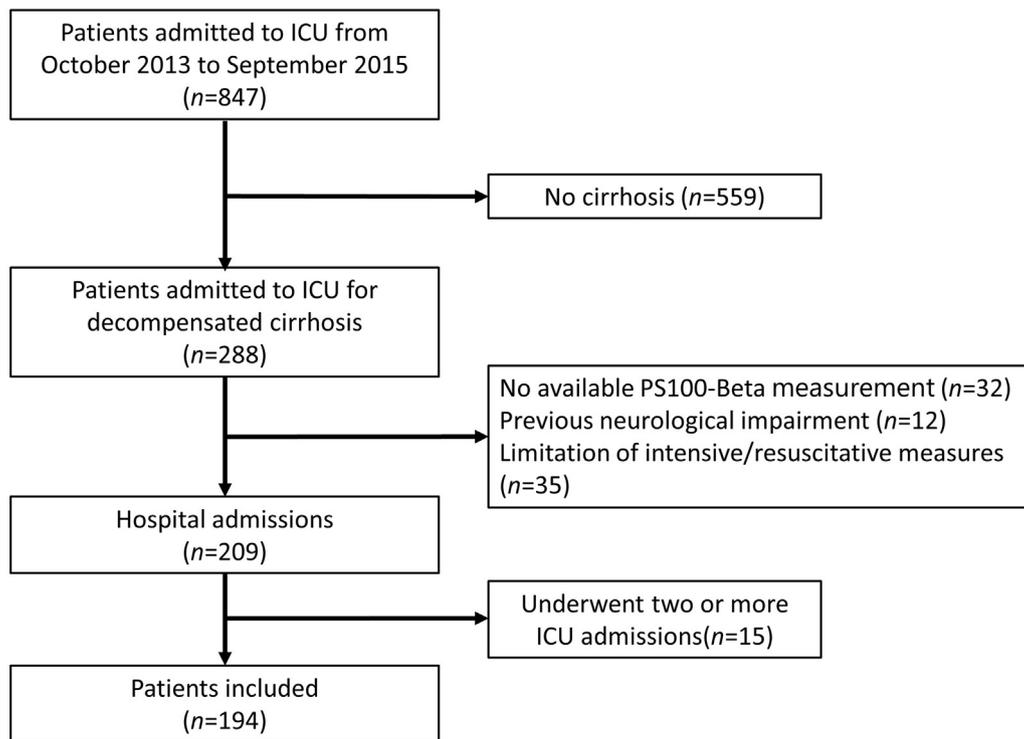
Figure 1. Flowchart of the patient inclusion and exclusion process.
ICU: Intensive care unit; PS100-Beta: Protein-S-100 Beta.

Biochemistry

ICU patients and outpatients underwent our standard protocol, which encompasses determination of sodium level, creatinine, albumin, ammonemia, hemoglobin, leukocytes, platelets, aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, prothrombin time, international normalized ratio (INR), factor V (FV), C-reactive protein (CRP), and procalcitonin (PCT). In ICU patients, lymphocyte and neutrophil counts were determined to calculate the lymphocyte-to-neutrophil ratio, which is a surrogate marker of systemic inflammation in cirrhotic patients.[25]

PS100-Beta determination

Samples of venous blood were collected in dry tubes from all patients at admission and at each patient's discharge from ICU if a blood sample was ordered for another medical reason. Serum PS100-Beta concentration was assayed on a Modular E170® analyzer (Roche Diagnostics, Mannheim, Germany), using an electrochemiluminescence immunoassay[18,19,26] characterized by a measuring range from 0.005 μg/L to 39 μg/L with cut-off value at 0.10 μg/L, and intra- and inter-assay coefficients of variation (CV) ≤3 %. The reaction involves two antibodies (Ab): a biotinylated Ab and a ruthenium-labeled Ab. The biotinylated Ab allows the attachment to a solid support (through a streptavidin–biotin connection). Normal PS100-Beta values are <0.10 μg/L.

Ammonia determination

Arterial or venous samples were collected in ethylene diamine tetraacetic acid (EDTA) tubes at admission and at each patient's discharge from ICU if a blood sample was ordered for another medical reason. Samples were placed immediately on ice and taken to the clinical laboratory within 30 min of collection, and were then centrifuged at 1885 *g* for 10 min.[27] Plasma ammonia concentration was measured on a Modular P800® analyzer (Roche Diagnostics) using an enzymatic kinetic method with a final photometric measurement of produced nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) at 340 nm, a measuring range from 5.87 μmol/L to 587.00 μmol/L, reference values from 11 μmol/L to 48 μmol/L for women and 15 μmol/L to 55 μmol/L for men, and intra- and inter-assay CV ≤8%.

Follow-up and outcome

After ICU discharge, the decompensated cirrhotic patients were followed by one of the senior hepatologists (i.e., planned follow-up every 3 months in our cohort). The occurrence of HE within 3 months after discharge, as well as overall survival and transplant-free survival, were retrieved from medical records. No specific follow-up of outpatients was planned.

Statistical analysis

Continuous variables are expressed as mean±standard error of the mean. Categorical variables are expressed as frequencies and percentages. Chi-squared or Fisher's exact test was used to compare categorical variables and analysis of variance (ANOVA) was used for continuous variables. To evaluate the ability of PS100-Beta – in combination with other factors – to predict overt HE, we performed an exploratory multivariate analysis using logistic regression. Three models were compared: (1) a

Table 1
Baseline characteristics of the patients.

Characteristics	Outpatient clinic patients (n=207)	ICU decompensated cirrhotic patients (n=194)	P-value
Age	56±1	58±1	0.134
Male	122 (58.9)	142 (73.2)	0.003
Etiology of liver disease			<0.001
Virus	48 (23.2)	9 (4.6)	
Alcohol	8 (3.9)	101 (52.1)	
NASH	72 (34.8)	25 (12.9)	
Mixed	52 (25.1)	45 (23.2)	
Other	27 (13.0)	14 (7.2)	
Previous conditions			
Child-Pugh	NA	10.2±0.2	NA
MELD	NA	20.5±0.5	NA
HCC	NA	27 (13.9)	NA
TIPS	NA	44 (22.6)	NA
Cause of ICU admission*			
Variceal bleeding	NA	61 (31.4)	NA
SBP	NA	6 (3.1)	NA
HE	NA	37 (19.1)	NA
AAH	NA	25 (12.9)	NA
Infection	NA	70 (36.1)	NA
Shock	NA	25 (12.9)	NA
Severity			
GCS	NA	14.0±0.2	NA
CLIF-C OF score	NA	5.9±0.1	NA
CLIF-C ACLF score	NA	78.7±0.6	NA
West-Haven score			
0	NA	92 (47.4)	NA
1	NA	28 (14.4)	NA
2	NA	47 (24.2)	NA
3	NA	18 (9.3)	NA
4	NA	8 (4.1)	NA
Biology			
Sodium (mmol/L)	141±0	135±1	<0.001
Creatinine (µmol/L)	77±1	100±5	<0.001
Albumin (g/L)	44±0	28±1	<0.001
Ammonemia (µmol/L)	35±1	81±3	<0.001
Hemoglobin (g/dL)	14.1±0.1	9.7±0.2	<0.001
Leukocytes (/mm^3)	6173±297	8985±372	<0.001
Lymphocytes/neutrophil ratio	NA	0.22±0.01	NA
Platelets (/mm^3)	217,541±4342	120,484±6403	<0.001
AST (IU/L)	41±2	136±24	<0.001
ALT (IU/L)	44±2	82±22	0.076
Bilirubin (µmol/L)	11±1	132±12	<0.001
PT (s)	92±1	48±1	<0.001
INR	1.1±0.1	1.9±0.0	<0.001
FV	113±2	56±2	<0.001
CRP (mg/L)	6±0	25±2	<0.001
PCT (µg/L)	0.05±0	1.13±0	<0.001
PS100-Beta (µg/L)	0.08±0	0.15±0.01	<0.001

Data are expressed as mean±standard error of the mean, n (%).
AAH: Acute alcoholic hepatitis; ACLF: Acute-on-chronic liver failure; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CLIF-C: Chronic liver failure consortium; CRP: C-reactive protein; FV: Factor V; GCS: Glasgow coma scale; HCC: Hepatocellular carcinoma; HE: Hepatic encephalopathy; ICU: Intensive care unit; INR: International normalized ratio; MELD: Model for end-stage liver disease; NA: Not available; NASH: Non-alcoholic steatohepatitis; OF: Organ failure; PCT: Procalcitonin; PS100-Beta: Protein-S-100-Beta; PT: Prothrombin time; SBP: Spontaneous bacterial peritonitis; TIPS: Transjugular intrahepatic portosystemic shunt.
* Patients could present several complications of cirrhosis at ICU admission.

model including MELD as the sole factor; (2) a model including ammonemia, PS100-Beta, PCT, and MELD; and (3) a model including age, ammonemia, PS100-Beta, PCT, MELD, presence of TIPS, and sodium levels. Likelihood ratios were used to determine the effects and odds ratios (OR) were calculated. The area under the receiver operating curve (AUROC) was used to estimate the diagnostic value of the three models. Survival analysis was performed by using the Kaplan–Meier method and survival between groups was compared using a Log-rank test. $P < 0.05$ (two-sided) was considered statistically significant. Statistical analyses were performed using JMP Pro-v16.0 (SAS Inst., Cary, NC, USA).

Results

Baseline characteristics

From October 2013 to September 2015, 209 hospital ICU admissions for decompensated cirrhosis with an available plasmatic measurement of PS100-Beta were identified (Figure 1). Among them, 15 patients underwent two or more ICU admissions. Thus, 194 patients were included in the ICU decompensated cirrhosis group. A total of 207 outpatients were identified and included in the final outpatient clinic group. Baseline characteristics of the 194 ICU patients and the 207 outpatients are
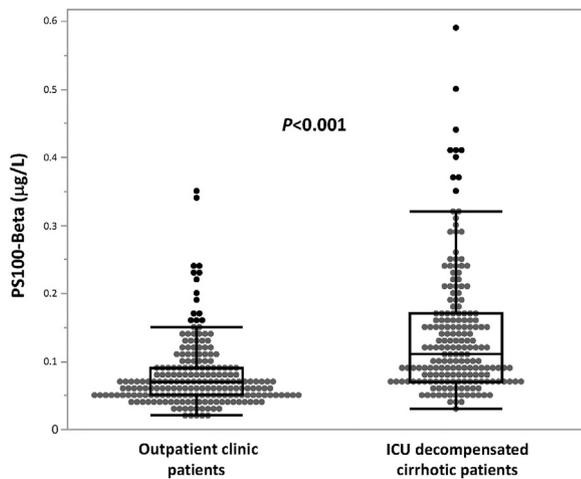
Figure 2. PS100-Beta in ICU decompensated cirrhotic patients compared to outpatients. Two outlier patients with very high values of PS100-Beta were excluded from the representation in this figure (PS100-Beta at 1.28 µg/L and 1.32 µg/L).
ICU: Intensive care unit; PS100-Beta: Protein-S-100 Beta.

Figure 3. PS100-Beta in outpatients whether they had F 0–3 or F 4 fibrosis and in ICU decompensated cirrhotic patients whether they had HE (West-Haven 2-4) or not (West-Haven 0-1). Two outlier patients with very high values of PS100-Beta were excluded from the representation in this figure (PS100-Beta at 1.28 µg/L and 1.32 µg/L).
HE: Hepatic encephalopathy; ICU: Intensive care unit; PS100-Beta, Protein-S-100 Beta.

shown in Table 1. Overt HE was present in 73 patients (38 %) at admission.

PS100-Beta

ICU decompensated cirrhotic patients had elevated levels of PS100-Beta compared with outpatients ([0.15±0.01] µg/L vs. [0.08±0] µg/L, $P < 0.001$) (Table 1 and Figure 2). These levels exceeded the upper limit of normal values (0.10 µg/L). Detailed values of other biochemical measurements for outpatients and ICU patients are also shown in Table 1. ICU patients also exhibited higher plasmatic levels of ammonemia ([81±3] µmol/L vs. [35±1] µmol/L) and PCT ([1.13±0] µg/L vs. [0.05±0] µg/L) ($P < 0.001$, respectively). PS100-Beta levels did not differ in outpatients between patients with F 0–3 ($n=178$) and those with F 4 ($n=29$) fibrosis ([0.08±0] µg/L vs. [0.08±0.01] µg/L, respectively, $P=0.670$) (Figure 3). Ammonemia was slightly higher in patients with F 4 compared with those with F 0–3 ([45±4] µmol/L vs. [34±1] µmol/L, respectively, $P < 0.001$) as was PCT ([0.07±0.01] µg/L vs. [0.05±0] µg/L, respectively, $P < 0.001$).

Prognostic value of PS100-Beta for overt HE and survival

Patients with overt HE (West-Haven 2-4) had higher levels of PS100-Beta compared with patients without overt HE (West-Haven 0-1) ([0.19±0.03] ug/L vs. [0.13±0.01] µg/L, $P=0.003$) (Table 2 and Figure 3). The patients with overt HE also had higher levels of ammonemia ([102±6] µmol/L vs. [69±4] µmol/L, $P < 0.001$) and PCT ([1.73±0.56] µg/L vs. [0.74±0.15] µg/L, $P=0.043$). Correlations between PS100-Beta levels and the different variables are shown in Figure 4. PS100-Beta was correlated with Child-Pugh score ($P < 0.001$), MELD ($P=0.004$), CRP ($P < 0.001$), ammonemia ($P < 0.001$), CLIF-C OF score ($P < 0.001$), and CLIF-C ACLF score ($P=0.038$), but not with leukocytes ($P=0.053$), PCT ($P=0.107$), or lymphocyte-to-neutrophil ratio in the subgroup of ICU patients ($P=0.522$).

Only 91 patients could be evaluated for the occurrence of bouts of overt HE in the 3 months following ICU discharge; PS100-Beta levels were not different in the patients displaying at least one episode of overt HE compared with those who did not ($P=0.169$).

Diagnostic value of PS100-Beta for overt HE diagnosis

The multivariate analysis for the prediction of overt HE in ICU patients for the three different models and their corresponding OR are given in Supplemental Table S1. Among the three models that were tested for the prediction of overt HE in ICU (model 1: MELD; model 2: ammonemia, PS100-Beta, PCT, and MELD; model 3: age, ammonemia, PS100-Beta, PCT, MELD, presence of TIPS, and sodium levels), model 3 had the best prediction with an AUROC of 0.765 (Figure 5).

Survival analysis and corresponding Kaplan–Meier curves are shown in Figure 6. Patients with a PS100-Beta level <0.12 µg/L had a better overall survival ($P=0.019$) and a better survival without liver transplantation ($P=0.013$).

Discussion

According to our results, ICU patients with decompensated cirrhosis had increased levels of PS100-Beta compared with outpatients; these values were higher than upper normal values. Furthermore, ICU patients with decompensated cirrhosis and displaying overt HE had significantly higher PS100-Beta levels compared with the patients without overt HE. The values did not appear to be correlated with the occurrence of overt HE within 3 months after ICU discharge. In multivariate analysis, PS100-Beta was independently associated with the diagnosis of overt HE in ICU patients with decompensated cirrhosis. The models including at least ammonemia, PS100-Beta, and PCT had the best AUROC. In addition, patients with a PS100-Beta level <0.12 µg/L had both a better overall survival and a better survival without liver transplantation.

PS100-Beta is a small dimeric cytosolic protein synthesized in astrocytes and Schwann cells. Increased serum lev-
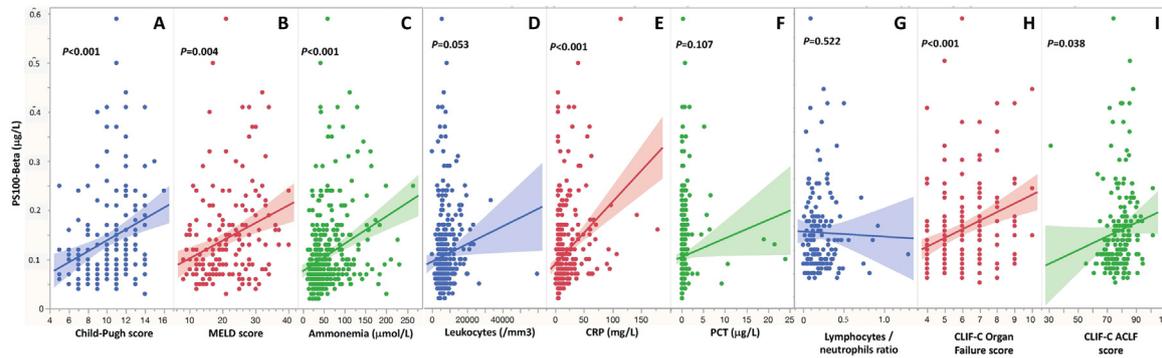
Figure 4. Correlation of PS100-Beta and markers of liver disease, inflammation, and severity. Correlation between PS100-Beta and Child-Pugh score (A), MELD score(B), ammonemia(C), leukocytes(D), CRP(E), PCT (F), lymphocyte-to-neutrophil ratio (G), CLIF-C organ failure score (H), and CLIF-C ACLF score (I).
ACLF: Acute-on-chronic liver failure; CLIF-C: Chronic liver failure consortium; CRP: C-reactive protein; MELD: Model for end-stage liver disease; PCT: Procalcitonin; PS100-Beta: Protein-S-100 Beta.
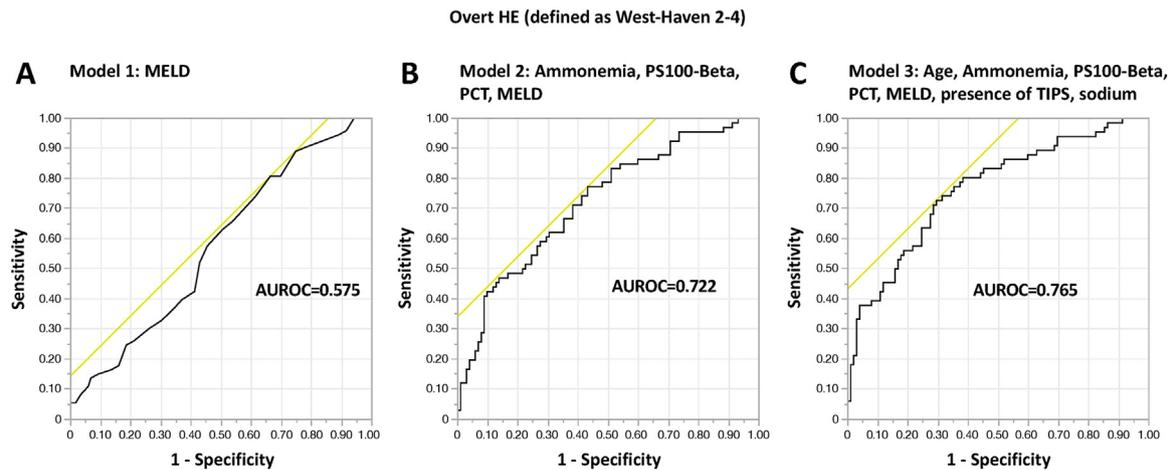
Figure 5. AUROC for the different multivariate models for the prediction of overt HE in the ICU. Model 1 includes MELD (A); Model 2 includes ammonemia, PS100-Beta, PCT, and MELD (B); Model 3 includes age, ammonemia, PS100-Beta, PCT, MELD, presence of TIPS, and sodium (C).
AUROC: Area under the receiver operating curve; HE: Hepatic encephalopathy; ICU: Intensive care unit; MELD: Model for end-stage liver disease; PCT: Procalcitonin; PS100-Beta: Protein-S-100 Beta; TIPS: Transjugular intrahepatic portosystemic shunt; WH: West-Haven score.
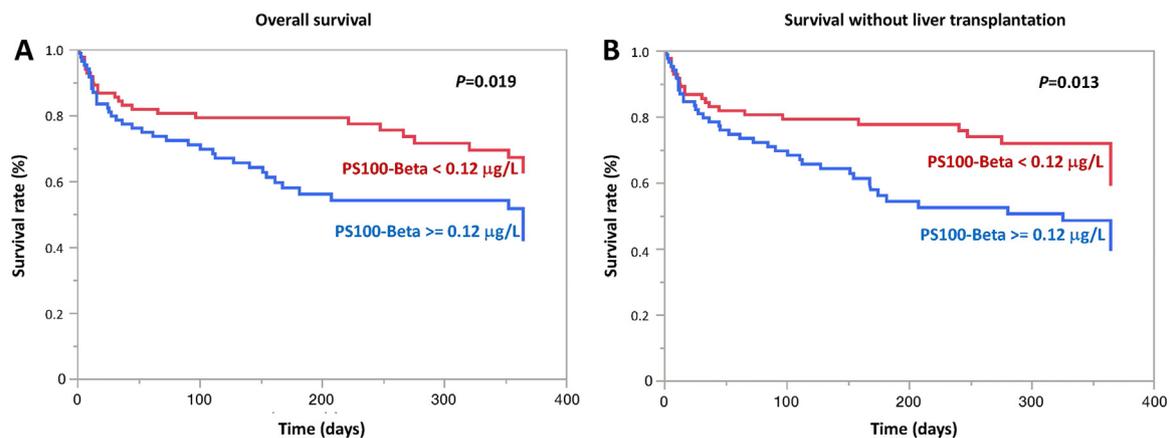
Figure 6. Kaplan–Meier survival curves according to the level of PS100-Beta. Overall survival according to the level of PS100-Beta, below or above 0.12 µg/L (A). Survival without liver transplantation according to the level of PS100-Beta, below or above 0.12 µg/L (B).
PS100-Beta: Protein-S-100 Beta.

Table 2
Characteristics of the patients according to clinical status and outcome.

Characteristics	Outpatient clinic patients			ICU decompensated cirrhotic patients		
	Fibrosis F 0–3(n=178)	Fibrosis F 4(n=29)	P-value	WH 0–1(n=120)	WH 2–4(n=73)	P-value
Age	54±1	67±2	<0.001	57±1	59±1	0.128
Male	103 (57.8)	19 (65.5)	0.453	88 (73.3)	53 (72.6)	0.912
Etiology of liver disease			<0.001			0.681
Virus	62 (34.8)	6 (20.7)		19 (15.8)	11 (15.1)	
Alcohol	90 (50.6)	9 (31.0)		63 (52.5)	39 (53.4)	
NASH	63 (35.4)	9 (31.0)		17 (14.2)	8 (11.0)	
Mixed	NA	3 (10.3)		21 (17.5)	16 (21.9)	
Other	26 (14.6)	11 (37.9)		16 (13.3)	7 (9.5)	
Previous conditions						
Child-Pugh	NA	NA		9.6±0.2	11.2±0.2	<0.001
MELD	NA	NA		19.8±0.7	21.8±0.9	0.070
HCC	NA	NA		19 (15.8)	8 (11.0)	0.409
TIPS	NA	NA		31 (25.8)	13 (17.8)	0.243
ICU admission*						
Variceal bleeding	NA	NA		51 (42.5)	10 (13.7)	<0.001
SBP	NA	NA		3 (2.5)	3 (4.0)	0.936
HE	NA	NA		0	37 (50.7)	<0.001
AAH	NA	NA		14 (11.7)	11 (15.1)	0.498
Infection	NA	NA		32 (26.7)	38 (52.1)	<0.001
Shock	NA	NA		13 (10.8)	12 (16.4)	0.251
Severity						
GCS	NA	NA		15±0	12±0	<0.001
CLIF-C OF score	NA	NA		5.3±0.1	6.8±0.2	<0.001
CLIF-C ACLF score	NA	NA		76.3±0.6	82.4±1.0	<0.001
Biology						
Sodium (mmol/L)	141±0	141±0	0.916	134±1	137±1	0.093
Creatinine (μmol/L)	77±2	79±4	0.755	101±7	99±8	0.840
Albumin (g/L)	44±0	42±1	0.181	29±1	27±1	0.122
Ammonemia (μmol/L)	34±1	45±4	<0.001	69±4	102±6	<0.001
Hemoglobin (g/dL)	14.1±0.1	14.4±0.3	0.350	9.7±0.3	9.8±0.2	0.712
Leukocytes (/mm^3)	6400±339	4784±309	0.057	8574±448	9613±653	0.177
Platelets (/mm^3)	229,085±3977	147,483±12,920	<0.001	122,678±7994	115,384±10,736	0.582
AST (IU/L)	40±2	49±4	0.102	115±17	172±59	0.261
ALT (IU/L)	43±3	51±6	0.277	66±10	107±56	0.373
Bilirubin (μmol/L)	10±1	17±2	<0.001	121±13	152±24	0.223
PT (s)	93±1	82±2	<0.001	50±2	44±2	0.013
INR	1.1±0.1	1.2±0.0	0.898	1.8±0.1	2.0±0.1	0.027
FV	115±2	95±5	<0.001	57±2	53±3	0.309
CRP (mg/L)	6±0	5±0	0.676	23±2	29±3	0.135
PCT (μg/L)	0.05±0	0.07±0.01	<0.001	0.74±0.15	1.73±0.56	0.043
PS100-Beta (μg/L)	0.08±0	0.08±0.01	0.670	0.13±0.01	0.19±0.03	0.003
Discharge						
Ammonemia (μmol/L)	NA	NA		73±12(n=32)	72±5(n=40)	0.912
PCT (μg/L)	NA	NA		0.6±0.2, (n=28)	0.9±0.4, (n=30)	0.471
PS100-Beta (μg/L)	NA	NA		0.14±0.02, (n=32)	0.17±0.04, (n=40)	0.558
Outcome						
HE at 3 months	NA	NA		17 (26.6),(n=64)	16 (48.4), (n=33)	0.032
Death	NA	NA		36 (33.3), (n=108)	29 (41.4),(n=70)	0.274
Liver transplantation	NA	NA		23 (19.2), (n=120)	13 (17.8), (n=73)	0.814

Data are expressed as mean±standard error of the mean, n (%).

AAH: Acute alcoholic hepatitis; ACLF: Acute-on-chronic liver failure; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CLIF-C: Chronic liver failure consortium; CRP: C-reactive protein; F: Fibrosis at FibroTest; FV: Factor V; GCS: Glasgow coma scale; HCC: Hepatocellular carcinoma; HE: Hepatic encephalopathy; ICU: Intensive care unit; INR: International standardized ratio; MELD: Model for end-stage liver disease; NA: Not available; NASH: Non-alcoholic steatohepatitis; OF: Organ failure; PCT: Procalcitonin; PS100-Beta: Protein-S-100-Beta; PT: Prothrombin time; SBP: Spontaneous bacterial peritonitis; TIPS: Transjugular intrahepatic portosystemic shunt; WH: West-Haven score.

* Patients could present several complications of cirrhosis at ICU admission.

els of PS100-Beta (normal values <0.10 μg/L) are the consequence of astrocytic dysfunction associated with altered functioning of the BBB, which explains the presence of PS100-Beta in the blood.[16,17] Several lines of evidence have suggested that PS100-Beta could constitute a potentially useful peripheral marker of BBB permeability.[13–17] Increased serum levels of PS100-Beta have been reported in subarachnoid hemorrhage,[18,19] traumatic brain injury including mild contusion,[22] ischemic and hemorrhagic strokes,[20] cardiac arrest,[21] epilepsy and status epilepticus,[13,23] and in cerebral tumors.[17]

Determination of serum levels of PS100-Beta thus offers the possibility to assess the BBB permeability at the bedside. We found evidence of increased levels of PS100-Beta in ICU patients with decompensated cirrhosis and this increase was even higher in the patients who also presented with overt HE. These results clearly suggest an increased BBB permeability in these patients and especially in those displaying overt HE. This finding is in accordance with our previous finding in similar patients by other techniques. Using a quantitative computed tomography scan, we previously reported that ICU cirrhotic patients

had impaired BBB functioning compared with healthy controls, and that among the ICU patients, those having HE had further increases in BBB permeability.[10] Using metabolomics in cirrhotic HE patients and animal models of chronic liver disease with HE, we could confirm impaired BBB permeability in those conditions.[9,11]

PS100-Beta values were correlated with the severity of the liver disease assessed by Child-Pugh, MELD, CLIF-C OF, and CLIF-C ACLF scores. Except for CRP, no correlation was found between PS100-Beta levels and any systemic inflammatory marker, leukocytes, PCT, or lymphocyte-to-neutrophil ratio (a surrogate marker of systemic inflammation in cirrhotic patients).[25] The positive correlation of PS100-Beta with CLIF-C OF and ACLF scores also suggests that PS100-Beta elevation could be due to the severity of the acute dysfunction. The reason for the correlation between PS100-Beta and ammonemia is unclear, but it could be partially explained by an effect of hyperammonemia or systemic inflammation on BBB permeability.[3–6] Multivariate analysis showed that PS100-Beta levels associated with ammonemia and PCT were independently predictive of the diagnosis of overt HE.

In addition to its role in HE pathophysiology, PS100-Beta might constitute – in association with other biomarkers encompassing ammonia – a diagnostic tool for overt HE. Indeed, in cerebral concussion, several studies suggested that PS100-Beta could be an excellent biomarker to identify cerebral lesion(s) due to mild concussion.[22,26] Nevertheless, other neurological diseases that can constitute a differential diagnosis of overt HE can display elevated PS100-Beta. However, many of these diagnoses, especially stroke or post-traumatic subarachnoid hemorrhage, can be diagnosed with brain imaging. A multimodal approach encompassing PS100-Beta measurement could be of interest. Even if additional prospective data in a larger cohort of cirrhotic patients in ICU with neurological impairment are needed, PS100-Beta might help to distinguish overt HE from toxic metabolic encephalopathy, which is a frequent differential diagnosis. Furthermore, our data on survival suggest that PS100-Beta could be used to either prioritize patients for specific HE treatment or perform an objective follow-up after their initiation.

Our survival analysis demonstrated a markedly better outcome for patients who had a PS100-Beta plasma concentration <0.12 μg/L at admission. A similar prognostic value of PS100-Beta was found in subarachnoid hemorrhage, where mean PS100-Beta values over the first days were prognostic of the outcome.[18,19] It cannot be excluded that PS100-Beta levels merely reflect the disease severity at admission, and this should be studied more in detail.

Some limitations should be noted. PS100-Beta is used as a cellular marker in pathology owing to its expression in some cancer cells. Thus, patients with sarcoma or melanoma can display unusually elevated levels of PS100-Beta.[28,29] Nevertheless, a recent study showed that extracranial sources of PS100-Beta were not able to significantly modify serum levels.[30] Therefore, it is likely that the serum levels of PS100-Beta found in our study are most probably of cerebral origin. Another limitation in the usefulness of these results is the accessibility of the measure. This dosage is, however, now standardized and can be implemented in most biochemistry departments.

Conclusions

Serum levels of PS100-Beta are elevated in ICU patients with decompensated cirrhosis, and even more so in those who also have overt HE. This suggests an increase in the permeability of the BBB in those patients. In addition to its use in pathophysiology studies of HE, PS100-Beta could constitute a potential biomarker that may facilitate positive or negative diagnosis of overt HE and help evaluate the treatment response in patients with HE.

Author Contributions

Nicolas Weiss: Formal analysis, Methodology, Project administration, Supervision, Writing – review & editing. **Simona Tripon:** Conceptualization, Data curation, Writing – original draft. **Maxime Mallet:** Data curation, Investigation, Writing – review & editing. **Françoise Imbert-Bismut:** Methodology, Validation, Writing – review & editing. **Mehdi Sakka:** Methodology, Validation, Writing – review & editing. **Dominique Bonnefont-Rousselot:** Methodology, Validation, Writing – review & editing. **Philippe Sultanik:** Data curation, Investigation, Writing – review & editing. **Sarah Mouri:** Data curation, Investigation, Writing – review & editing. **Marika Rudler:** Conceptualization, Methodology, Data curation, Investigation, Writing – review & editing. **Dominique Thabut:** Conceptualization, Methodology, Supervision, Writing – review & editing, Validation, Resources.

Ethics Statement

Samples were collected after written informed consent, in accordance with the local ethics committee of La Pitié-Salpêtrière Hospital (institutional committee), Paris, France (Comité de Protection des Personnes 33_13, 24th April 2013).

Conflict of Interest

Nicolas Weiss declares having perceived consultant fees from MedDay Pharmaceuticals and Owkin. Dominique Thabut declares having perceived consultant fees from MedDay Pharmaceuticals and Alfasigma. Other authors have nothing to disclose.

Data Availability

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

Supplementary Materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jointm.2023.08.006.

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