Modification in CSF specific gravity in acutely decompensated cirrhosis and acute on chronic liver failure independent of encephalopathy, evidences for an early blood-CSF barrier dysfunction in cirrhosis

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ABSTRACT

Introduction: Although hepatic encephalopathy (HE) on the background of acute on chronic liver failure (ACLF) is associated with high mortality rates, it is unknown whether this is due to increased blood-brain barrier permeability. Specific gravity of cerebrospinal fluid measured by CT is able to estimate blood-cerebrospinal fluid-barrier permeability. This study aimed to assess cerebrospinal fluid specific gravity in acutely decompensated cirrhosis and to compare it in patients with or without ACLF and with or without hepatic encephalopathy.

Method: We identified all the patients admitted for acute decompensation of cirrhosis who underwent a brain CT-scan. Those patients could present acute decompensation with or without ACLF. The presence of hepatic encephalopathy was noted. They were compared to a group of stable cirrhotic patients and healthy controls. Quantitative brain CT analysis used the Brainview software that gives the weight, the volume and the specific gravity of each determined brain regions. Results are given as median and interquartile ranges and as relative variation compared to the control/baseline group.

Results: 36 patients presented an acute decompensation of cirrhosis. Among them, 25 presented with ACLF and 11 without ACLF; 20 presented with hepatic encephalopathy grade$\geq$2. They were compared to 31 stable cirrhosis patients and 61 healthy controls. Cirrhotic patients had increased cerebrospinal fluid specific gravity (CSF-SG) compared to healthy controls (+0.4%, $p<0.0001$). Cirrhotic patients with ACLF have decreased CSF-SG as compared to cirrhotic patients without ACLF (-0.2%, $p=0.0030$) that remained higher than in healthy controls. The presence of hepatic encephalopathy did not modify CSF-SG (-0.09%, $p=0.1757$). Specific gravity

did not differ between different brain regions according to the presence or absence of either ACLF or HE.

Conclusion: In patients with acute decompensation of cirrhosis, and those with ACLF, CSF specific gravity is modified compared to both stable cirrhotic patients and healthy controls. This pattern is observed even in the absence of hepatic encephalopathy suggesting that blood-CSF barrier impairment is manifest even in absence of overt hepatic encephalopathy.

Key words: hepatic encephalopathy; brain edema; blood-brain barriers; blood-cerebrospinal fluid-barrier; CT scan

INTRODUCTION

Overt hepatic encephalopathy (HE) requiring hospital admission is a common complication of cirrhosis that is associated with high resource utilization and a significant in-hospital mortality [1,2]. HE patients have a significantly lower survival compared with patients without HE at all severities of liver disease [3,4]. Acute on chronic liver failure (ACLF) is a clinically and prognostically distinct syndrome that occurs in cirrhotic patients who are hospitalized with acute deterioration due to either known or unknown precipitating events, hepatic and/or extrahepatic organ failure and high short term mortality rates. Pathophysiologically, the syndrome is characterized by altered host response to injury. One major recent finding is that HE in patients displaying a diagnosis of ACLF have higher mortality compared with HE patients without ACLF [3].

The mechanism underlying the pathophysiology of HE in ACLF and this susceptibility to increased mortality is not known. In spite of its high frequency, HE pathophysiology is poorly understood. Increased cerebral ammonia uptake, and its transformation into glutamine by the astrocytes are thought to be important mechanisms causing neurological symptoms [5]. However, since there is poor correlation between ammonia and the severity of HE, other factors could be implicated [6,7]. For instance, the cerebral accumulation of different substances besides ammonium, like amino acids (AA) [8], manganese [9], biliary salts, has been demonstrated in patients with cirrhosis or porto-systemic shunts. More recently, inflammation has been thought to contribute to the severity of HE both in animal models and also in humans [6,10]. Indeed, inflammation is clearly more marked in patients with ACLF and HE compared with those with acute decompensation but no ACLF with similar severities of HE, suggesting that the underlying pathophysiological

mechanisms may be different. Severe cerebral edema has been reported in some studies in cirrhotic patients with HE [11]. Indeed, death of about 5% ACLF patients is thought to be caused by severe cerebral edema [12]. Whether this is associated with blood-brain barriers dysfunction is not known.

Brain homeostasis is of major importance to carry out the complex physiological functions, especially synaptic transmission. Thus, exchanges between the blood and the brain are tightly regulated by tight junctions on two main cellular interfaces that restrict the permeability: the blood-brain barrier that is located on cerebral endothelial cells and the blood-cerebrospinal fluid barrier (BCSFB) located on epithelial cells of choroid plexuses (see [13,23] for review). Even if tight junctions are expressed in the cerebral endothelial cells of the blood-brain barrier and on epithelial cells of choroid plexuses on the BCSFB, their molecular and cellular organization is very closely organized (see for review [13,14]). Dysfunction of these barriers have been recently highlighted in different neurological diseases and their dysfunction in cirrhotic patients, especially those with HE, has been suggested to be pathophysiologically relevant [11,14]. Recent studies highlighted that the BCSFB constitute a large surface area for exchanges, greater than previously thought, and that this barrier intervene in availability of drugs. Thus, MRI studies have shown that in case of manganese accumulation for example, this metal first accumulates in CSF as a transfer across the choroid plexuses before spreading to periventricular regions where it enter into neural cells to reach the final destination via retrograde transport via axons [24]. Therefore, a more precise analysis of CSF in cirrhotic patients with or without ACLF would be of major interest but access to CSF is difficult since these patients often have low platelets that render lumbar puncture a high risk procedure.

The measurement of the specific gravity (SG), which is defined as the weight of the volume of an object, is a validated and reliable method to characterize any solute, estimate the water content of CSF or of different brain regions [15,16]. CT scanning and post-processing analysis by a dedicated software, Brainview (Institut Nationale des Télécommunications [15,17,18]), has already been used to assess SG in patients for the evaluation of brain edema [15,19,20]. Thus, studying CSF-SG allows this question to be addressed. The normal CSF-SG is between 1.007 and 1.008. Disease related variations of SG could be analyzed in patients that undergo a CT scan [20]. A decrease in CSF-SG suggests passage through the BCSFB of either a substances with a SG lower than that of the CSF or the passage of solute whereas an increase of CSF-SG suggests the passage through the BCSFB of a substances with a SG greater than that of the CSF. Interestingly, SG of the substances that has previously been shown as being present in the CSF of cirrhotic patients are well known and are as follows, ammonium has a SG of 0.9, bile salts a SG between 1.010 and 1.040 and, immunoglobulins a SG of more than 1.060.

Thus, the modification in the blood-CSF barrier permeability could represent a first step in the physiopathology of ACLF or HE, responsible for modification in the CSF composition and afterwards in the brain extracellular compartment.

Therefore, the aims of this study were to assess CSF-SG in acutely decompensated cirrhosis and to compare it in patients with and without ACLF and with and without HE.

PATIENTS AND METHODS

Patient's selection

We retrospectively identified all the patients admitted for acute decompensation of cirrhosis in two Hepatology ICUs of two tertiary care centers (Royal Free Hospital, London, United-Kingdom and La Pitié-Salpêtrière Hospital, Paris, France) who underwent a brain CT-scan between January 2013 and April 2013. Both center admit about 250 patients every years for acute decompensation of cirrhosis and 50% of these patients present ACLF and 40% present with HE. Those patients could present acute decompensation with or without ACLF [21] and with or without HE. Patients were included, provided they fulfilled the following criteria: (1) CT-scan with contiguous slices without interspaces; (2) no previous neurological history except HE; (3) no cerebral hemorrhage on CT; (4) no history of contrast medium injection within the last 2 weeks for analysis purpose. Patients were compared to a group of stable cirrhotic patients followed up in the same units who underwent brain CT-scan in their pre-transplant workup for hepatocellular carcinoma in order to rule out brain metastasis. Cirrhosis was diagnosed using a combination of clinical, biological, radiological, histological and anatomo-pathological features.

Healthy controls

Non-cirrhotic patients that underwent a CT-scan with contiguous slices without interspaces at emergency department for headaches or trauma and that were finally interpreted as normal were used as healthy controls.

Data collection

The following data were collected at admission: demographic data (age, gender),

natural history of cirrhosis (etiology, presence of ascites, previous episodes of bleeding, previous episodes of HE, hepatocellular carcinoma), cause of admission, physical examination, laboratory measurements (leukocyte and platelet count, prothrombin time (PT), international normalized ratio (INR), and levels of liver enzymes, bilirubin, creatinine, sodium, albumin and C-reactive protein (CRP)). The severity of cirrhosis was evaluated by the Child-Pugh score. The presence of HE and of ACLF was noted. The presence of HE was diagnosed as impairment in consciousness, or motor function in a patient with cirrhosis after exclusion of other causes of mental disturbance. The severity of HE was assessed by senior hepatologists using West-Haven score (WH). Overt HE was considered if WH was equal or higher than 2. To quantify organ dysfunction, the CLIF-OF score was used. This score derived from SOFA assess six organ systems (liver, kidneys, brain, coagulation, circulation, lungs) and takes in account the specificity of cirrhosis [21]. The presence or the absence and grade of ACLF was defined as previously described [22].

CT-scan analysis

CT-scans were acquired on a Philips scanner (Netherlands) in London and on a Siemens scanner (Germany) in Paris. Quantitative brain CT analysis was performed using the Brainview software (Institut National des Télécommunications) [17]. Briefly, this validated software [17,18] is aimed to analyze DICOM images acquired from cerebral CT scans by performing automatical segmentation excluding extracranial compartments on each slices. Interactive slice-by-slice segmentation allows to delineate different anatomical regions. The software renders the weight, the volume

and the specific gravity (SG) of the whole brain and of each individual region (right and left hemispheres, brainstem, cerebellum, and intraventricular CSF).

Statistical analysis

Continuous variables are expressed as median and interquartile range (IQR). Categorical variables are expressed as frequencies and percentages. Chi-square or Fisher exact test was used to compare categorical variables and ANOVA for continuous variables, with Tukey range test for correction. Univariate analysis was used to compare the variables between the different groups. In order to take in account the acquisition in two centers, and the possible differences in CT calibration, stratification according to the center was performed.

All statistical tests were two-tailed. *P* values that were less than 0.05 were considered to indicate statistical significance. Analysis involved use of JMP v9.0 (SAS Inst., Cary, NC).

According to the French law, the approval of the local ethics committee was not necessary since the CTs were included in the clinical management of the patients and the study was retrospective. The database in which the patients were identified had been submitted to and approved by the French national commission for computerized files and liberty (CNIL, Commission Nationale de l'Informatique et des Liberte´s). Royal Free Hospital research ethics committee gave its approval for the study.

RESULTS

Subjects

From January 2013 to April 2013, 72 cirrhotic patients underwent a cerebral CT scan. Five CTs were not analyzable, thus 67 patients were finally retained for analysis. Overall, 36 cirrhotic patients were admitted for acute decompensation (25 presented with ACLF and 11 with acute decompensation without ACLF) and 31 for stable cirrhosis. Results were compared to that of 61 healthy controls. Baseline characteristics of the patients and the controls are displayed in Table 1. In patients with acute decompensation, 20 patients (56%) displayed overt HE (WH grade 2: n=12, grade 3, n=7, grade 4, n=1) and 25 (69%) displayed ACLF. 15 patients (42%) presented with both ACLF and HE. No patients had HE or ACLF in the stable cirrhotic patients. Indication for brain CT-scan differed significantly between patients with and without overt HE. Brain CT-scans in healthy controls were performed mainly because of headache.

Quantitative CT analysis

SG values did not differ between the two centers. Thus, data were analyzed together.

Cirrhotic patients whether they were or not decompensated display increased CSF-SG as compared to healthy controls

Compared to healthy controls, cirrhotic patients had increased CSF-SG (1.01178 [1.01014-1.01375] vs 1.00855 [1.00688-1.01045], +0.3 %, $p<0.0001$, respectively) suggesting the passage of substances with a SG greater than that of the CSF through the BCSFB in cirrhotic patients.

Acutely decompensated cirrhotic patients display a mild decrease in CSF-SG as compared to patients with stable cirrhosis

Acutely decompensated cirrhotic patients compared to stable cirrhotic patients had a decreased CSF-SG (1.01088 [1.00922-1.01224] vs 1.01256 [1.01156-1.01458] respectively, -0.2%, p=0.0002) suggesting increased permeability of the BCSFB to either solutes or substances with a lower SG in this setting (Table 2 and Figure 1A). CSF-SG in acutely decompensated cirrhotic patients remained however higher than in healthy controls (1.01088 [1.00922-1.01224] vs 1.00855 [1.00688-1.01045], respectively, +0.2%, p<0.0001).

Cirrhotic patients with ACLF have decreased CSF-SG as compared to cirrhotic patients without ACLF

Cirrhotic patients with ACLF displayed decreased CSF-SG as compared to patients without ACLF (1.01036 [1.00908-1.01207] vs 1.01218 [1.01103-1.01412] respectively, -0.2%, p=0.0030,), suggesting increased permeability of the BCSFB to either solutes or substances with a lower SG in this setting (Table 3 and Figure 1B).

The presence of HE does not modify the CSF-SG in cirrhotic patients.

In cirrhotic patients, patients with HE displayed a CSF-SG of 1.01122 [1.00986-1.01224] compared to 1.01209 [1.01024-1.01408], -0.09%, in patients without HE (p=0.1757) (Table 3 and Figure 1C).

DISCUSSION

By using CT scanning analysis of the SG of the CSF, we found arguments in favor of increased permeability of the BCSFB in cirrhotic patients. Indeed, results of the present pilot study suggest that cirrhotic patients display alterations of the BCSFB, that are dependent on the severity of liver disease. Importantly, these alterations are influenced by the presence of ACLF, but occur even in the absence of HE. These results suggest that, in cirrhotic patients, BCSFB permeability to substances and solutes are altered.

It seems that, early in cirrhosis, even in those without acute decompensation, some substances with a SG higher than that of the CSF, i.e. higher than 1.008, cross the BCSFB. Elevated levels of glutamine, glutamate, alpha ketoglutarate, bile salts and increased concentrations of serotonin, dopamine and AA have been reported in the CSF of cirrhotic patients [26–28]. According to our results in stable cirrhotic patients compared to healthy controls, the passage of bile salts, the SG of which ranges from 1.010 to 1.040, into the CSF could explain the increase in CSF-SG. Indeed, bile salts have been shown to accumulate in these conditions [29]. Furthermore, bile salts have a synergistic effect with endotoxin and ammonia on the formation of brain edema by their action on Na-K-ATPase [30]. Several data also reported an increased level in both brain and CSF of tryptophan and its metabolites 5-HIAA, in patients with porto-systemic shunts and cirrhosis [31]. Hence, in cirrhotic patients the level of tryptophan in the CSF was 4-fold higher than in controls. Several AA were found to be elevated in the CSF in the setting of ALF [32], and animal models of cirrhosis displayed an increased CSF level of tryptophan [31]. In the CSF of cirrhotic patients without HE, a significant increase was found in nearly all amino acids, including those

known not to cross the blood-brain barriers readily, suggesting the presence of a nonspecific modification of the BCSFB permeability [28]. This could also explain the increase of CSF-SG in the cirrhotic patients. The passage of proteins such as IgG seems less probable since no major brain edema was present. The possible passage of some yet unknown substance or medication cannot be ruled out. Indeed, increase of CSF-SG is a classical finding in the administration of local anesthesics for spinal anaesthesia. In some pathological conditions such as seizures, increased blood osmolarity can induce dehydration of the brain and thus an increase its SG [33]. We recently showed using metabolomics that the CSF of cirrhotic patients displaying neurological symptoms contained the main above mentioned substances and several xenobiotics, i.e. drugs, that all could contribute to the modification in CSF-SG [34,35].

In cirrhotic patients with HE, CSF-SG was not statistically different compared to cirrhotic patients without HE. This observation is somewhat surprising given that ammonia (SG: 0.9) has been shown to be increased in the CSF in patients with HE [36]. The data support the hypothesis that alterations of cerebral homeostasis occur even in the absence of HE, evidenced by studies showing alterations in myoinositol and glutamine in the brain of patients with cirrhosis with and without HE [37,38]. The manifestation of HE is therefore likely to be due not only to alterations of blood-brain barriers, but also to other interacting factors.

In cirrhotic patients with ACLF, CSF-SG was decreased compared to acute decompensation without ACLF patients. This finding suggests again an alteration in BCSFB. Inflammation is the main physiopathological hallmark of ACLF. Hence, one hypothesis is an increased passage of solutes since BCSFB permeability to solutes,

is particularly influenced by inflammatory mediators such as TNF-alpha or IFN-gamma. Our results suggest that ACLF patients present altered BCSFB even in the absence of HE. The significance of this observation is not clear. It is tempting to hypothesize that these abnormalities may make the patient susceptible to the development of HE but this hypothesis will have to be confirmed in future studies.

Our study has several limitations. First, it was retrospective and the diagnosis of ACLF and HE was retrieved in medical records. Bias could be introduced in our study by the fact that we considered only overt HE and not minimal HE. Since this retrospective design, case-mix cannot be excluded. For example, patients who underwent a CT-scan for another reason than HE were patients hospitalized for the workup of hepatocellular carcinoma, which were displayed far more severe liver disease than usual patients with hepatocellular carcinoma. Furthermore, we could include only patients that underwent CTs without interslice spaces. Last, the composition of substances that cross the BCSFB need further analysis of the CSF but lumbar puncture is rarely indicated in cirrhotic patients, and this type of study is probably not ethically possible. This study provides the novel data describing changes in CSF content in cirrhotic patients.

In conclusion, our study describes for the first time that CSF-SG is modified in cirrhotic patients, both with and without HE suggesting altered BCSFB permeability to substances and solutes. Patients with ACLF display further alterations of BCSFB, which may indicate their susceptibility to HE. The observation that abnormalities in the BCSFB are manifest even in the absence of overt HE may have important clinical and pathophysiological implications.

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List of abbreviations

ACLF	Acute-on chronic liver failure
AA	Amino-acids
ALF	Acute liver failure
BCSFB	Blood-cerebrospinal fluid barrier
CSF	Cerebrospinal fluid
CRP	C-reactive protein
HE	Hepatic encephalopathy
INR	International normalized ratio
MRI	Magnetic resonance imaging
MELD	Model for End-Stage Liver Disease
PT	Prothrombin time
SG	Specific gravity
WH	West-Haven score

Conflict of interest

Nicolas Weiss declares the following potential conflicts of interest.
Research grant: Eumedica, Gilead
Speaker fees: Alpha-Wasserman, Norgine, Gore

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Rajiv Jalan declares the following potential conflicts of interest. Inventor: Ornithinephenylacetate, a treatment for hepatic encephalopathy; UCL Liver dialysis device. Research grant: Ocera therapeutics, Gambro, Sequana and Grifols. Consultant: Ocera therapeutics, Conatus. Founder: Yaqrit ltd. (UCL spin out). Speaker fees: Grifols, Norgine.

The other authors have nothing to declare.

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Table 1: Baseline characteristics of the patients and the controls.

	Acute decompensation of cirrhosis	Stable cirrhotic patients	Healthy controls	p value
	n = 36	n=31	n = 61	
Age, years	55 [47-65]	59 [48-63]	64 [46-78]	0.3347
Male gender (%)	23 (64%)	26 (84%)	39 (64%)	0.1134
Etiology of cirrhosis				
Viral	4 (11%)	0 (0%)	-	
Alcohol consumption	22 (61%)	22 (72%)	-	
Other	10 (28%)	8 (25%)	-	
Ascites	9 (56%)	0 (0%)	-	**< 0.0001**
ACLF	25 (69%)	0 (0%)	-	**< 0.0001**
Hepatocelullar carcinoma	3 (8%)	31 (100%)	-	**< 0.0001**
Acute alcoholic hepatitis	6 (38%)	0 (0%)	-	0.0003
TIPS	7 (44%)	-	-	
Asterixis	11 (69%)	0 (0%)	-	**< 0.0001**
PTT (%)	32 [31-44]	64 [46-76]	NA	**0.0001**
INR	2.0 [1.6-2.5]	1.4 [1.2-2.0]	NA	**0.0081**
Leukocyte count (/mm3)	9 625 [6 683-16 848]	4 900 [3 450-7 800]	10 030 [7140-12 920]	**0.0055**
Platelet count (/mm3)	81 500 [50 500-125 750]	67 000 [47 500-88 500]	NA	0.3442
Creatinine level (mg/dL)	0.94 [0.63-1.35]	0.74 [0.67-0.89]	NA	0.1309
Albumin level (g/L)	25 [22-31]	34 [31-40]	NA	**0.0014**
Bilirubin level (mg/dL)	5.1 [1.7-8.9]	1.1 [0.8-1.9]	NA	**0.0013**
AST level (U/L)	82 [60-177]	67 [46-82]	NA	0.0931
ALT level (U/L)	48 [25-80]	45 [30-55]	NA	0.3700
Sodium level (mmol/L)	139 [133-145]	139 [134-142]	NA	0.3339
CRP level (mg/l)	20.5 [6.3-48.3]	7.5 [4.0-14.0]	NA	0.0203
Child-Pugh score				
A	2 (6%)	26 (83%)	-	
B	2 (6%)	5 (17%)	-	
C	32 (88%)	0	-	
CLIF-SOFA score	11 [8-12]	NA	-	
Hepatic encephalopathy	20 (56%)	0 (0%)		**< 0.0001**
West-Haven score				**< 0.0001**
0	4 (11%)	31 (100%)	-	
1	12 (33%)	-	-	
2	12 (33%)	-	-	
3	7 (19%)	-	-	
4	1 (3%)	-	-	

Data are mean ± SD or number (%).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; HE, hepatic encephalopathy; INR, international standardized ratio; MELD, Model for End-Stage Liver Disease*; PTT, prothrombin time*

Table 2: Specific gravity of different brain regions according to the presence or not of cirrhosis and the presence or not of acute decompensation of cirrhosis.

	Healthy controls n = 61	**Stable cirrhotic patients** n = 31	**Acute decompensation of cirrhosis** n = 36	**p-value**
CSF	1.00855 [1.00688-1.01045]	1.01256 [1.01156-1.01450]	1.01088 [1.00922-1.01224]	**< 0.0001**
Relative difference (%)	na	+ 0.4 %	+ 0.2 %	
Whole brain	1.02934 [1.02812-1.03108]	1.02920 [1.02792-1.03023]	1.02844 [1.02708-1.02967]	**0.0329**
Relative difference (%)	na	- 0.01 %	- 0.08 %	
Hemispheres	1.02903 [1.02753-1.03058]	1.02885 [1.02766-1.02965]	1.02793 [1.02657-1.02940]	**0.0462**
Relative difference (%)	na	- 0.01 %	- 0.1 %	
Brainstem	1.02138 [1.02080-1.02231]	1.02534 [1.02373-1.02593]	1.02448 [1.02290-1.02580]	**< 0.0001**
Relative difference (%)	na	+ 0.4 %	+ 0.3 %	
Cerebellum	1.03502 [1.03310-1.03619]	1.03297 [1.03081-1.03360]	1.03201 [1.03114-1.03430]	**< 0.0001**
Relative difference (%)	na	- 0.3 %	- 0.3 %	

Relative differences are given compared to controls.
Abbreviation: CSF, cerebrospinal fluid; na: not applicabla.

Table 3: Specific gravity of different brain regions according to the presence or absence of ACLF and HE.

	Hepatic encephalopathy			**ACLF**		
	No n = 47	**Yes** n = 20	**p-value**	No n = 42	**Yes** n = 25	**p-value**
CSF	1.01209 [1.01024-1.01408]	1.01122 [1.00986-1.01224]	0.1757	1.01218 [1.01103-1.01412]	1.01036 [1.00908-1.01207]	**0.0030**
Relative difference (%)	Na	- 0.09 %		na	- 0.2 %	
Whole brain	1.02940 [1.02782-1.03023]	1.02854 [1.02718-1.02966]	0.4353	1.02912 [1.02781-1.03014]	1.02866 [1.02669-1.02975]	0.1450
Relative difference (%)	na	- 0.08 %		na	- 0.05 %	
Hemispheres	1.02871 [1.02737-1.02968]	1.02812 [1.02663-1.02942]	0.3946	1.02873 [1.02747-1.02965]	1.02838 [1.02613-1.02960]	0.1255
Relative difference (%)	na	- 0.06%		na	- 0.03 %	
Brainstem	1.02494 [1.02342-1.02571]	1.02487 [1.02305-1.02630]	0.7815	1.02515 [1.02344-1.02572]	1.02479 [1.02292-1.02618]	0.6563
Relative difference (%)	na	- 0.01%		na	- 0.04 %	
Cerebellum	1.03266 [1.03083-1.03360]	1.03256 [1.03130-1.03455]	0.3952	1.03268 [1.03079-1.03359]	1.03226 [1.03122-1.03443]	0.6648
Relative difference (%)	na	- 0.01%		na	- 0.04 %	

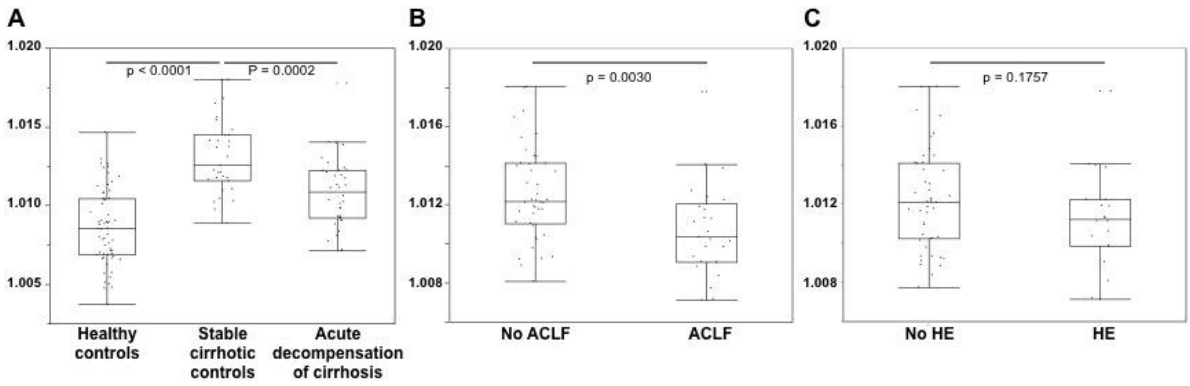
Relative differences are given compared to controls.
Abbreviation: ACLF, acute on chronic liver failure ; CSF, cerebrospinal fluid.

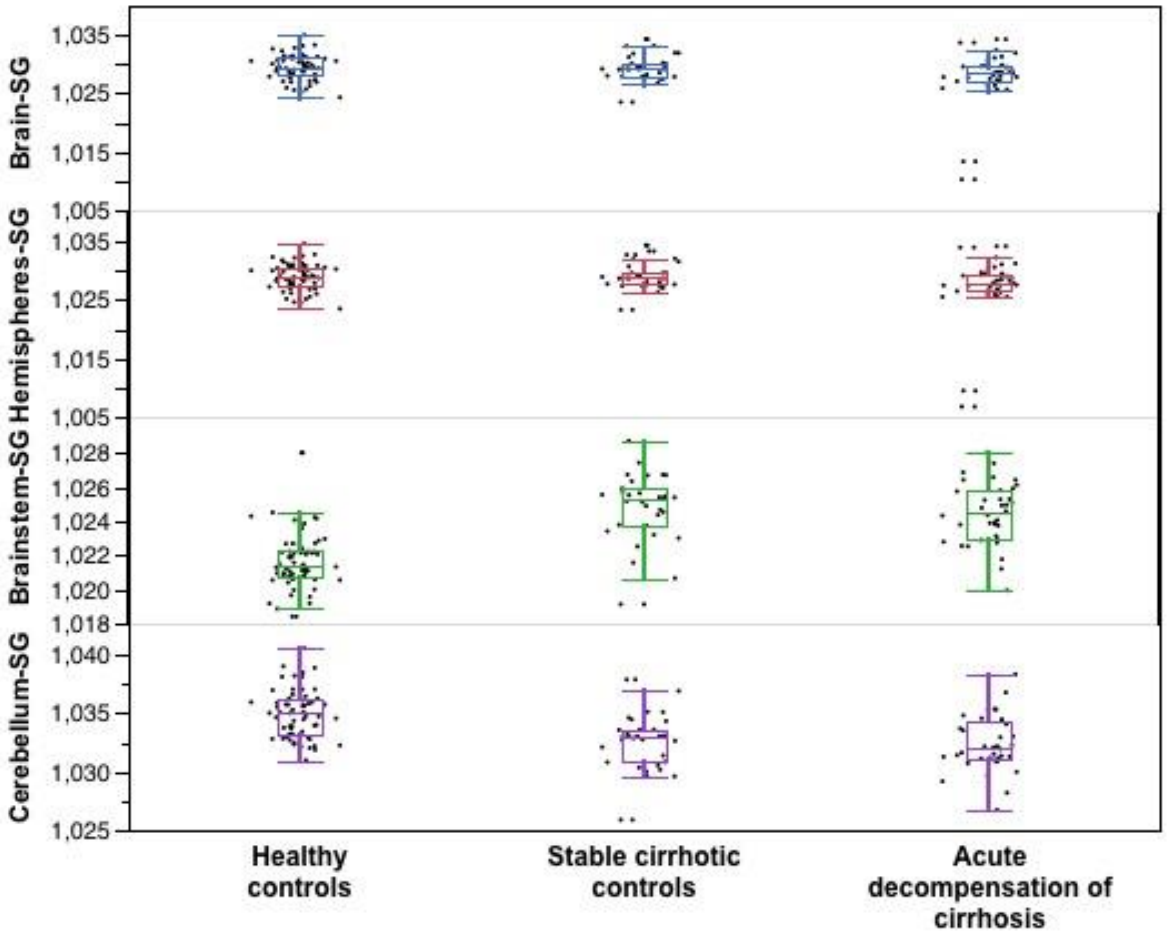
FIGURES LEGENDS

Figure 1: CSF specific gravity according to the presence or not of cirrhosis, the presence or not of acute decompensation of cirrhosis, of ACLF and HE.

A, CSF SG according to the presence or not of cirrhosis and the presence or not of acute decompensation of cirrhosis; B, CSF SG according to the presence or not of ACLF; C, CSF SG according to the presence or not of HE.

Supplemental figure: Specific gravity of different brain regions according to the presence or not of cirrhosis and the presence or not of acute decompensation of cirrhosis.


A
B
C
p < 0.0001
P = 0.0002
p = 0.0030
p = 0.1757
Healthy controls
Stable cirrhotic controls
Acute decompensation of cirrhosis
No ACLF
ACLF
No HE
HE


Brain-SG
Brainstem-SG Hemispheres-SG
Cerebellum-SG
Healthy controls
Stable cirrhotic controls
Acute decompensation of cirrhosis