Genetic Fingerprint of Hepatic Encephalopathy Risk in Liver Cirrhosis

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BACKGROUND: To determine the impact of genetic factors on the development of hepatic encephalopathy (HE) in patients with liver cirrhosis.

METHODS: Patients suffering from compensated liver cirrhosis (n = 434; estimation cohort (n = 294) and validation cohort (n = 140)) were included. Patients were followed up for five years until HE bouts, liver transplant, or death. Methods: Patients were genotyped for 62 candidate SNPs (genes involved in the pathophysiology of HE: inflammation, ammonia and glutamine metabolism, intestinal barrier integrity and oxidative stress) by using OpenArray custom plates. Likewise, a haplo-type formed by four SNPs within GLS plus the length of a microsatellite in the promoter region of GLS were determined (Romero-Gómez et al. Ann Intern Med 2010). Statistical analysis was performed by Cox regression and Kaplan-Meier for continuous and categorical data. Significant variables, and those known as weighted prognostic indicators, were entered into multivariable models by competing risks, according to Fine and Gray's method.

RESULTS: In the estimation cohort, competing risks analysis showed GLS mutations, FUT2-alleles at risk, three levels of risk patients were defined: low, mid or high risk (sHR: 1; 6.5 (1.8–22.9) P = 0.004; 27.1 (7.5–96.8) P < 0.001, respectively) (C-index = 0.82). This regression model performed in a similar manner in the validation cohort (sHR: 1; 4.2 (1.2–14.3) P = 0.024; 10.0 (2.7–36.7) P < 0.001] (C-index = 0.78). Cumulative survival free of HE after 5 years was also influenced by this genetic fingerprint: 95.3%, 77.0% and 42.5% for the low, mid and high-risk groups (log-Rank 53.1, P < 0.001) in the estimation, and 85.2%, 56.0% and 40.0% (log-Rank 14.1; P < 0.001) in the validation cohort, respectively (Figure 1).

CONCLUSIONS: Combination of unfavorable variants could predict HE. This genetic fingerprint could be implemented in clinical practice for decision making in the management of cirrhotic patients. Besides, this work emphasizes the role of these pathways in the pathophysiology of HE and brings out novel genes as potential therapeutic targets.
Clinical Value of Asterixis in a Large Population of Well-characterised Patients With Cirrhosis

P: 3 Junior Investigator | Oral Presentation

Clinical Value of Asterixis in a Large Population of Well-characterised Patients With Cirrhosis and Varying Degree of Hepatic Encephalopathy

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BACKGROUND: Current guidelines for the diagnosis of overt hepatic encephalopathy (HE) include the clinical sign asterixis, which has also been used as an outcome in clinical trials. Limited information is available on the ability of physicans to detect/grade asterixis, and the relationship between asterixis and other HE indices. The aim of the study was to retrospectively assess the clinical value of asterixis in a large population of well-characterised patients with varying degree of HE.

METHODS: Asterixis was sought for/graded in 374 consecutive patients with cirrhosis (54 ± 8 yrs, 280 males, MELD 14 ± 5; Pugh 7.9 ± 2.0) by trainees in Internal Medicine (n = 16), attending the Padova HE clinic between 2011 and 2019. Patients were asked to stretch their arms, extend their wrist and spread their fingers, and observed for 1 min. Asterixis was qualified as absent, rare, frequent or continuous. All underwent neuropsychiatric examination as per local protocols, including electroencephalography (EEG) and a set of neuropsychological tests (Animal Naming Test (ANT), Psychometric Hepatic Encephalopathy Score (PHES), computerized simple (sRT), choice (cRT) and Scan (sCANT) reaction times). Laboratory indices (venous ammonia, CRP, sodium) were recorded, together with procedures and medications from 1 year before cirrhosis diagnosis to 3 months after cirrhosis diagnosis. Opioid exposure was assessed over a 6 month period after cirrhosis diagnosis. Exposure to gabapentin and statins was assessed in the subsequent 6 months (i.e. 6 months-1 year after cirrhosis diagnosis). We performed multivariable logistic regression, adjusting for Charlson comorbidity index, cirrhosis etiology, sex and age to assess the association between opioid, gabapentin and statin prescription and development of complications.

RESULTS: Of all patients with compensated cirrhosis (n = 7,580), 30.3%, 2.6% and 10.0% received prescriptions for opioids, gabapentin and statins within 6 months after cirrhosis diagnosis. In the subsequent 6 months, 3.1% developed HE and its diagnosis was more common among those with prescriptions for opioids than those without (4.2% vs 2.6%, P = 0.001) but not associated with prescriptions for gabapentin (4.1% vs 3.0%, P = 0.397) or statins (2.8% vs 3.1%, P = 0.738). In the multivariable model, a higher odds of HE was seen in patients with opioid prescriptions (OR 1.69, 95% CI 1.36). In multivariable models, opioids were not significantly associated with other HE indices. Positive associations were observed between patients with different degrees of asterixis. Some were prominent between patients with the sign, regardless of its frequency (PHES, ScanRT), while for others the relationship was linear (ANT, sRT, sCANT, spectral EEG parameters). Significant differences in neuropsychometric HE indices were observed between patients with different degrees of asterixis. Some were prominent between patients with/without the sign (Phug, CRP), while for others the relationship was linear (MELD, ammonia). Finally, the likelihood of developing HE-related hospitalisations over the follow up period was significantly higher in patients with asterixis (Cox-Mantel = P = 0.028).

CONCLUSIONS: Asterixis is reliably detected/graded by specialists in a tertiary referral liver centre, and shows significant associations with established neuropsychological, neurophysiological and laboratory HE indices, as well as the likelihood of developing HE-related hospitalisations over time.
Mice injected with AOM had increased Let7f and decreased IGF1 expression in the brain. However, whether Let7f contributes to the development of HE is unknown. The aims of this study were to assess the expression of Let7f in a mouse model of Type A HE and to determine its involvement in the neurological complications of acute liver failure (ALF).

METHODS: C57Bl/6 mice were injected with azoxymethane (AOM) to induce ALF and HE. In parallel, mice were given an intracerebroventricular infusion of a Let7f antagonist or recombinant IGF1 (rIGF1) for 3 days prior to AOM injection. Cognitive impairment was monitored by reflex response assessment at various time points. Neuroumuncular deficits were assessed using a grip strength meter, and a digit span analysis system was utilized to measure ataxia. Liver damage was assessed by hematoxylin and eosin staining and serum chemistry. IGF1, Let7f and proinflammatory cytokine expression were assessed by immunohotting, immunohistochemistry and/or qPCR. Microglia were stained by IBA1 and cortex cytokine expression were assessed by immunoblotting, immunohistochemistry and/or qPCR. Mouse neurons were transfected with a Let7f mimic and treated with vehicle or rIGF1 for 4 to 24 hr. The expression and secretion of IGF1 and the proinflammatory chemokine, CCL2, was assessed by qPCR and EIA.

RESULTS: Mice injected with AOM had increased Let7f and decreased IGF1 expression in the frontal cortex. Treatment with a Let7f antagomir attenuated the i) suppression of cortical IGF1, ii) neuroinflammation, and iii) neurological and neuromuscular deficits of AOM-treated mice. Specific targeting of IGF1 expression by Let7f was demonstrated in vitro, where treatment of neurons with a Let7f mimic suppressed IGF1 expression and secretion. Furthermore, treatment of neurons with Let7f mimic increased the expression of CCL2, which could be attenuated with the co-treatment with rIGF1. Lastly, infusion of rIGF1 to restore the dampened IGF1 signaling attenuated the neurological and neuroinflammatory deficits, as well as the neuroinflammation observed in AOM-treated mice.

CONCLUSIONS: Elevated cortical Let7f expression contributes to the pathogenesis of HE in AOM-treated mice via mechanisms involving the suppression of IGF1 expression. These deleterious effects of Let7f during HE can be reversed by inhibiting Let7f expression or by increasing IGF1 concentration in the brain.

BACKGROUND: Patients with cirrhosis are growing older and the overlap between hepatic encephalopathy (HE) & pre-dementia mild cognitive impairment (MCI) is unclear. HE affects visuospatial/psychomotor speed while MCI affects memory. Aim: Determine the performance of elderly cirrhotics on tests for HE and dementia and their impact on quality of life (QOL).

METHODS: Outpatient cirrhotics and controls ages 65–95 years were recruited at 4 centers. Subjects had to have MMSE > 25, no current HE or dementia to be eligible. All subjects got tests for HE (psychometric hepatic encephalopathy score, PHES: 3 tests & low score = poor, EncephalApp, OffTime + OnTime, high score = poor) and QOL (Sickness Impact profile(SIP), high score = poor QOL). Cirrhotics also got tests for MCI, RBANS (tests immediate memory, delayed memory, language, visuospatial & attention). A neuro-psychologist evaluated results and divided cirrhotics into (A) unimpaired (B) MCI only (C) HE only & (D) MCI/HE overlap. Demographics, MELD, alcohol etiology, and SIP were compared. Finally, age, gender and education-adjusted norms were created for PHES and EncephalApp based on the controls. Presence of MHE and sensitivity of EncephalApp for MHE diagnosis were evaluated.

RESULTS: 169 cirrhotics and 108 non-cirrhotic subjects were included. Demographics/education levels were statistically similar between centers. Controls were older than cirrhotics (74.9 ± 6.6 vs 70.5 ± 4.4, P < 0.05) and had similar education/gender distribution. Despite this, controls performed better than cirrhotics on all tests (PHES 2.9 ± 12.4 vs -4.5 ± 4.5, P < 0.001, EncephalApp Off + On 101.4 ± 71.9 vs 218.2 ± 88.0, P = 0.03) and had a better QOL (SIP total 3.8 ± 7.1 vs 7.9 ± 9.5, Psych 3.1 ± 8.4 vs 7.2 ± 11.1, Phys 3.0 ± 6.2 vs 6.7 ± 9.5, all P < 0.01). Within cirrhotic subgroups (Table 1), demographics, MELD/alc & MMSE were similar. Pts with both MHE & HE had worse cognition on all tests, which translated into a worse QOL compared to other groups. Presence of HE, with/without MCI, contributed towards poor QOL. Norms for PHES/EncephalApp: Adjusting for age, gender & education, 17% (n = 71) patients were positive for MHE on PHES compared to controls. On EncephalApp, 49% (n = 47) patients were positive. AUC for EncephalApp using PHES as the gold standard was 0.86 (0.78-0.95 CI).

CONCLUSIONS: In this multi-center study, adjusted norms defining the high sensitivity of EncephalApp to diagnose HE in older individuals were created. Presence of HE regardless of MCI contributed towards poor cognition and QOL in patients >65 years.
In this prospective study, we de…

**CONCLUSION:**

In our study, we demonstrated that the EncephApp, di…

**CONCLUSIONS:**

In this prospective study, we define the performance of diagnostic codes for the identification of HE in the electronic health record study. Medications specific for HE therapy outperformed diagnostic codes.

### P: 8  Junior Investigator | Oral Presentation

**Assessment of Cirrhotic Patients With Covert Hepatic Encephalopathy (HE) Through the EncephApp (Stroop-Test) Based on Critical Flicker Frequency and PHES-Test**

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**BACKGROUND:** In daily clinical practice, the detection of HE is still less represented but strongly required. Therefore, we assessed the EncephApp (Stroop-Test) in a German population by standard diagnostic procedures such as the Critical Flicker Frequency (CFF) and the PHES-Test (Psychomotor Hepatic Encephalopathy Score). One of the purposes of the trial was finding a Cut-Off value for the EncephApp in the German population.

**METHODS:** 81 patients with liver cirrhosis underwent the testing of the CFF, the PHES-Test and the EncephApp. A control group of 25 healthy subjects were examined in the same manner. The CFF was considered pathological with <0.39 Hz and the PHES-Test with < -4 value points. The On-Off Time of the EncephApp was compared to the results of the CFF and the PHES-Test. Different Cut-Offs of the On-Off Time were analyzed. Within these HE groups, the mean values of the PHES-Test, the CFF and the On-Off Time of the EncephApp were compared and the ROC Analysis (receiver operating characteristic) was conducted. Laboratory parameters, clinical data, and further imaging techniques were also included and compared.

**RESULTS:** The study group (n = 81) included 52 men and 29 women (62.8 years ± 12.5). For the evaluation of the EncephApp, different Cut-Off values were determined and their specificity and sensitivity were calculated. The comparative parameter was the result of the PHES-Test. Through the creation of a ROC curve, the AUC (area under the curve) showed that the Cut-Off time of >724 sec with 0.74 was the most promising with better sensitivity (42.8%) and specificity (82%). Other Cut-Off values showed lower sensitivities and higher false negative values.

**CONCLUSIONS:** After evaluating the specificity, sensitivity and AUC the most efficient Cut-Off value for the On-Off Time in the EncephApp is > 224 sec. In comparison with the CFF and the PHES-Test, the majority of patient indentify better sensitivity compared to the CFF (82%) and the PHES-Test (77%). A significant difference was found in both patient groups after transplantation. The psychometric test battery consisted of the Poortsetyntom Encephalopathy Syndrome-Test which provides the psychometric hepatic encephalopathy score (PHES), the Repeatability Battery for the Assessment of Neuropsychological Status (RBANS), the Inhibitory Control Test and the Critical Flicker Frequency. All patients were asked to complete the self-reporting questionnaires Hospital Anxiety and Depression Scale, Beck Depression Inventory, Fatigue Impact Scale and Short Form 36 health survey to assess health-related quality of life. Patients were divided into 2 groups: Patients with and without hepatic encephalopathy before liver transplantation. Test results were compared to those of 55 adjusted healthy controls.

**METHODS:** 34 patients with liver cirrhosis on the waiting list for liver transplantation underwent a psychometric battery and questionnaires assessing health-related quality of life: 3.4 ± 3.1 months (T1) before liver transplantation. Further 33 patients were included directly after liver transplantation. After transplantation all patients (n = 67) underwent psychometric testing and completed questionnaires at 3.6 months (T2) and at 11.7 months (T3). The psychometric test battery consisted of the Poortsetyntom Encephalopathy Syndrome-Test which provides the psychometric hepatic encephalopathy score (PHES), the Repeatability Battery for the Assessment of Neuropsychological Status (RBANS), the Inhibitory Control Test and the Critical Flicker Frequency. All patients were asked to complete the self-reporting questionnaires Hospital Anxiety and Depression Scale, Beck Depression Inventory, Fatigue Impact Scale and Short Form 36 health survey to assess health-related quality of life.

**METHODS:** 34 patients with and without HE before transplantation were assessed using the psychometric tests: RBANS total mean 69.3 ± 15.4 and PHES total score 7.7 ± 8.6. The psychometric test battery consisted of the Poortsetyntom Encephalopathy Syndrome-Test which provides the psychometric hepatic encephalopathy score (PHES), the Repeatability Battery for the Assessment of Neuropsychological Status (RBANS), the Inhibitory Control Test and the Critical Flicker Frequency. All patients were asked to complete the self-reporting questionnaires Hospital Anxiety and Depression Scale, Beck Depression Inventory, Fatigue Impact Scale and Short Form 36 health survey to assess health-related quality of life.

**RESULTS:** Patients tested before liver transplantation performed significantly worse than controls in the psychometric tests: RBANS total mean 69.3 ± 15.4 and PHES total score 7.7 ± 8.6. The psychometric test battery consisted of the Poortsetyntom Encephalopathy Syndrome-Test which provides the psychometric hepatic encephalopathy score (PHES), the Repeatability Battery for the Assessment of Neuropsychological Status (RBANS), the Inhibitory Control Test and the Critical Flicker Frequency. All patients were asked to complete the self-reporting questionnaires Hospital Anxiety and Depression Scale, Beck Depression Inventory, Fatigue Impact Scale and Short Form 36 health survey to assess health-related quality of life. Patients were divided into 2 groups: Patients with and without hepatic encephalopathy before liver transplantation. Test results were compared to those of 55 adjusted healthy controls.

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analyzed cognition (EncephalApp; high = worse) at baseline and 30 days post-intervention (Figure 1a red arrow). Stool microbiota was analyzed using 16s rRNA & BAs using LC/MS. Fecal BA moieties analyzed were (a) total (b) primary (c) secondary (d) deconjugated (e) tertiary BAs. Secondary/primary BA ratios were calculated. Serum was also analyzed for lipopolysaccharide-binding protein (LBP) & IL-6. Correlation networks between BAs, microbiota, LBP, IL-6 and cognition were created. Correlation network complexity was compared between post-FMT vs post-placebo states.

RESULTS: All subjects completed the follow-up without any serious AEs related to FMT/placebo. EncephalApp total score (P < 0.05) improved in FMT pts only Microbiota: there was a significant enrichment of donor microbiota with higher Ruminococcaceae & Lachnospiraceae in stool/duodenum (Figure 1b,d). BAs: There was a significant increase in secondary/primary BA ratio (Figure 1c) in FMT pts. Deconjugation and tertiary BAs remained similar between groups. Correlation network showed higher complexity after FMT compared to place-control (Figure 1e). Beneficial bacteria (Ruminococcaceae and Verrucomicrobiaceae) became significantly positively correlated with each other (blue lines) and negatively with inflammation (IL6 redlines) and associated with better EncephalApp score post-FMT (Figure 1f) compared to placebo at study end.

CONCLUSIONS: Capsular FMT is safe and improves cognition in pts with cirrhosis and HE compared to placebo. These improvements are associated with beneficial changes in microbial composition and function and differential correlations with bacterial translocation and inflammation.

**CONCLUSIONS:**
1. Capsular FMT is safe and improves cognition in pts with cirrhosis and HE compared to placebo. These improvements are associated with beneficial changes in microbial composition and function and differential correlations with bacterial translocation and inflammation.
2. There was a significant increase in secondary/primary BA ratio (Figure 1c) in FMT pts. Deconjugation and tertiary BAs remained similar between groups. Correlation network showed higher complexity after FMT compared to placebo (Figure 1e). Beneficial bacteria (Ruminococcaceae and Verrucomicrobiaceae) became significantly positively correlated with each other (blue lines) and negatively with inflammation (IL6 redlines) and associated with better EncephalApp score post-FMT (Figure 1f) compared to placebo at study end.

**METHODS:** To date eighteen patients have been treated (FMT n = 13; placebo n = 5). 71% of those treated were male (mean age 54.4 years (range 38–75) and mean MELD 12.88 (range 10–16)). Patients continued lactulose, but were required to discontinue all antibiotics, including rifaximin, two weeks prior to treatment. Following bowel preparation with Moviprep™, 200 mL FMT/placebo was delivered via naso-jejunal tube in to the proximal jejunum at gastroscopy. Patients were assessed for adverse events and underwent clinical review at day 7, 30 and 90 with biobanking of stool, saliva, urine, whole blood and plasma for quantitative metagenomics, metabonomics, bacterial DNA quantification, bile acids, whole blood culture in response to endotoxin and plasma cytokines and leucocyte function testing at a later date.

**RESULTS:** The FMT has been well tolerated and the five serious adverse events (SAEs) reported requiring hospital admission (placebo n = 1; FMT n = 4) were not treatment-related. Biochemistry, leucocyte count and MELD score did not change. Mean venous ammonia reduced at day 30 in the FMT cohort [71.67 μmol/L (95% CI 54.43–88.9) day 0 versus 51.22 μmol/L (CI 35.18–67.26) day 30] whereas it increased in the placebo group [54.4 μmol/L (95% CI 38.17–70.63) day 0 versus 73.25 μmol/L (95% CI 22.54–124) at day 30]. Figure 1 shows a reduction in delta ammonia at day 7 [mean −1.15 (−16.23 to 13.73 95% CI)] versus day 30 [−15.11 (−2.82 to −27.4) post FMT].

**CONCLUSIONS:** The interim analysis shows that nasojejunal administered FMT appears safe and well tolerated in patients with advanced cirrhosis and reduces venous ammonia at day 30.

**Figure 1: Effect of treatments which specifically target ammonia on hepatic encephalopathy in cirrhosis**

**PROFIT: PROspective, Randomised Placebo-controlled Feasibility Trial of Faecal Microbiota Transplantation in Cirrhosis Interim Analysis**

Charlotte Woodhouse, MBBS1, Victoria Kronsten, MBBS1, Anz Zamalloa, RN1, Grace Hatton, MBBS1, Vishal C. Patel, MBBS1, Simon Goldberg, MBBS, MD2, Debbie L. Shawcross, MBBS, PhD1.

1King's College Hospital, London, UK; 2Guy's and St Thomas’ NHS Foundation Trust, London, UK.
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<tr>
<th>T2</th>
<th>Healthy controls (n=55/test)</th>
<th>HE (n/test)</th>
<th>NHE (n/test)</th>
<th>p value</th>
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<td><strong>PHES (25th ; 75th Percentile)</strong></td>
<td>1 (0.2)</td>
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<td>0.5 (-1.2)</td>
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<td>(n=30)</td>
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<td><strong>RBANS T6 (meansSD)</strong></td>
<td>99.9 ±12.0</td>
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<td>94.4 ±12.5</td>
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<td>(n=36)</td>
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<td><strong>RBANS Attention (25th ; 75th Percentile)</strong></td>
<td>103 (94;112)</td>
<td>89.5 (79;103)</td>
<td>98.5 (85;108.6)</td>
<td>0.001*</td>
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<td><strong>RBANS Delayed Memory (25th ; 75th Percentile)</strong></td>
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**T3**

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<tr>
<td><strong>RBANS T6 (meansSD)</strong></td>
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The Role of Monocarboxylate Transporter-1 and Lactate Metabolism on the Development of Cognitive Deficits During NAFLD

Anna Hadjihambi, PhD1, Patrick S Hosford, PhD2, Rajiv Jalan, PhD, MD3,4,5, Luc Pellerin, PhD1.

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BACKGROUND: Non-alcoholic fatty liver disease (NAFLD) is a major complication of obesity. Certain observations regarding NAFLD induced neuropsychiatric and neurochemical alterations have been reported but mechanisms are unknown (Seo, 2016). In this context, monocarboxylate transporter-1 (MCT1) haploinsufficient mice, which resist high fat diet (HFD) induced hepatic steatosis represent an interesting model (Carneiro, 2017). Using a mouse model of NAFLD (HFD followed by high fructose/high glucose in water [HF/HG]) we investigated the development of cognitive deficits and state of cerebral oxygenation and cerebrovascular reactivity.

METHODS: Behavioural tests (open field/novel object recognition/forced swimming test [FST]) were performed in mice fed control diet (NC, WT + NC, MCT1 ± + NC) or HFD HF/HG (WT + HFD HF/HG, MCT1 ± + HFD HF/HG) for 16 weeks. Baseline PO2 (in somatosensory cortex) and in response to systemic hypercapnia (10% CO2) was monitored under anaesthesia by a fluorescence method (Oxylite™). Microelectrode biosensors were used for measurements of lactate release by cortical slices. EchoMRI was performed to assess lean/fat mass.

RESULTS: Increased fat mass (not lean mass) was observed in WT and MCT1 ± mice (50% less) on HFD HF/HG compared to NC controls. Liver mass was only significantly higher in WT ± HFD HF/HG mice compared to NC controls. Behavioural tests did not reveal any significant differences between groups except for FST, which indicated a depression-related behaviour in the WT ± HFD HF/HG group compared to their controls. This was not observed with MCT1 ± HFD HF/HG mice. WT ± HFD HF/HG mice had a lower cerebral PO2 baseline and PO2 response induced by systemic hypercapnia compared to NC controls (although significance was not reached), while the MCT1 ± groups remained unchanged. Tonic lactate release was unaltered between all groups although the MCT1 ± HFD HF/HG group indicated a trend of decreased lactate tone.

CONCLUSIONS: Our results suggest that NAFLD is associated with a depression-related behaviour and a trend of decreased cerebral PO2 baseline. MCT1 haploinsufficient mice were resistant to the reported phenotypes, suggesting a link between liver metabolism and neuropathophysiological alterations in NAFLD.

| RBANS Attention (25th ; 75th Percentile) | 103 (94;112) | 97 (88;112) | 103 (86;115) | 0.3 |
| RBANS Delayed Memory (25th ; 75th Percentile) | 100.5 (94.5;100.5) | 103 (95;110) | 100.5 (96.5;110.5) | 0.23 |
| RBANS Visuospatial/Constructional (25th ; 75th Percentile) | 89 (81;109) | 89 (81;102) | 100 (84;112) | 0.15 |
| RBANS Immediate Memory (25th ; 75th Percentile) | 106 (94;112) | 103 (90;114) | 106 (90;114) | 0.87 |
| RBANS Language (25th ; 75th Percentile) | 101.5 (95.5;108) | 99 (92;110) | 98 (85;114) | 0.45 |
| CFF (25th ; 75th Percentile) | 44 (41.7;45.5) | 43 (40.1;46.8) | 43.9 (40.9;45.5) | 0.62 |
| ICT Targets (%) (25th ; 75th Percentile) | 98 (96.7;99.1) | 98 (94.100) | 99 (97.8;100) | 0.4 |

SD standard deviation, n number, HE Hepatic Encephalopathy, NHE No hepatic Encephalopathy, n number, SD standard deviation, PHES hepatic encephalopathy score, RBANS Repeatable Battery for the Assessment of Neuropsychological Status, TS Total Scale, CFF Critical Flicker Frequency, ICT Inhibitory Control Test, vs. versus, Con Controls, t1 before transplantation, t2 9 months after transplantation, t3 5 years after transplantation, * overall between groups, P value ≤0.05 is considered significant

[10]
Non-alcoholic Fatty Liver Disease Alters Expression of Genes Governing Hepatic Nitrogen Conversion

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BACKGROUND: We recently showed that the functional capacity for ureagenesis is deficient in patients with NAFLD. The aim of this study was to assess the expression of urea cycle and related genes to elucidate whether there may be a gene regulatory basis to the functional problem.

METHODS: Liver mRNA expression analyses within the gene pathway governing hepatic nitrogen conversion were performed in 20 non-diabetic, biopsy-proven NAFLD patients (8 simple steatosis; 12 non-alcoholic steatohepatitis (NASH)), and compared with 12 obese and 14 lean healthy control persons. The relationship between gene expressions and functional capacity for ureagenesis was described.

RESULTS: Gene expression of most urea cycle-related enzymes were downregulated in NAFLD vs. both control groups and markedly so for the urea cycle flux-generating carbamoyl phosphate synthetase (CPS1) (3.5-fold, \( P < 0.0001 \)). In the NASH patients, the reduction of CPS1 expression paralleled the deficit in functional ureagenesis \(( P = 0.03 )\). Additionally, the expression of several genes involved in amino acid uptake and degradation, and the glucagon receptor gene, were downregulated in NAFLD. Conversely, gene expression of glutamine synthetase (GS) increased more than 1.5-fold \(( P \geq 0.03 )\), inversely related to CPS1 expression \(( P \approx 0.004 )\).

CONCLUSIONS: NAFLD downregulated the expression of urea cycle-related genes. Downregulation of the urea cycle flux-generating enzyme CPS1 correlated with loss of functional capacity for urea synthesis in patients with NASH. On gene level, these changes coincided with an increase in the major ammonia scavenging enzyme GS. The effects seemed to be related to a fatty liver as such rather than NASH or obesity. The findings support that gene regulatory mechanisms are involved in the deficient urea synthesis of NAFLD, but it remains unexplained how the hepatocyte fat accumulation exerts these.
Results: Groups were matched for age, sex, baseline educational level, weekly alcohol consumption, baseline PHES score, Cogstate TM computerised psychometric testing, WTA and SF6 scores. Color-naming subtest of the Stroop task was significantly impaired at baseline in LOLA receivers ($P = 0.0179$) for group averaged whole-brain data; there was no significant difference in activation for both motor and cognitive tasks or in resting state in all 34 patients at baseline and 12 weeks. Group differences by region of interest (see Table 1 below) the choice reaction time task in the default mode network (DMN) demonstrated significant treatment ($P = 0.026$) advantage after 12 weeks of LOLA. Of the 12 resting state networks studied, visual area 2 showed a significant treatment ($P = 0.021$) benefit after 12 weeks of LOLA.

Conclusion: In the first RCT of LOLA combined with modern brain imaging analysis, a significant treatment benefit with LOLA on task activation was noted in the DMN. A significant treatment advantage of LOLA on resting-state fMRI in the visual network is consistent with this, this may be a compensatory mechanism in early MHE. Future studies could stratify patients who may benefit from LOLA based on baseline fMRI characterisation.

P: 16 Junior Investigator | Oral Presentation

Traditional Prognostic Tools are Superior to Cognitive Testing and Stool Frequency as Predictors of Poor Outcomes in Cirrhotic Patients Admitted with Hepatic Encephalopathy

Patricia Pringle Bloom, MD$^1$

$^1$Massachusetts General Hospital, Boston, MA, USA.

BACKGROUND: Model for end-stage liver disease-sodium (MELD-Na) score and extrahepatic organ failures (EHOFs) predict poor outcomes in cirrhotic patients, including those with hepatic encephalopathy (HE); however, there is a need for the development of additional and specific predictors for outcomes in HE. We aim to determine if cognitive testing, total daily lactulose dose (TDL), and stool frequency at hospital discharge predicts readmission and other poor outcomes in patients admitted with overt HE.

METHODS: We performed a prospective study of patients admitted to a single transplant center with overt HE. When the primary team anticipated discharge within 48 hours, consented subjects underwent Psychometric HE Score (PHES), Montreal Cognitive Assessment (MOCA), liver frailty index (LFI), and stool frequency assessment. MELD-Na, EHOFS, and other clinical variables were assessed via chart review. At 30 days post-discharge, subjects were called to evaluate for a composite primary outcome of HE readmission, transition to hospice, liver transplantation, or death. T-test or chi-square test compared predictors between those who did and did not meet the primary endpoint.

RESULTS: Of 175 potential candidates, 52 patients provided informed consent and enrolled, with the majority (82%) of participants excluded for discharge, death, transplant, or transfer to hospice before enrollment could be performed. Within 30 days, 7 patients had a readmission for HE, and 11 died, were transplanted or transferred to hospice. Seventeen patients met the primary composite endpoint. MELD-Na (28.1 ± 8.0 vs 22.0 ± 6.0, $P = 0.01$) and the number of EHOFS (1.3 ± 1.3 vs. 0.5 ± 0.8, $P = 0.03$) were higher in patients who met the primary composite outcome. Most predictors did not vary between those who met and did not meet the primary endpoint, including MOCA score ($P = 0.73$), PHES ($P = 0.97$), stool frequency ($P = 0.34$), total daily lactulose dose ($P = 0.80$), LFI ($P = 0.57$), admission ammonia ($P = 0.58$), or being discharged on rifaximin ($P = 0.70$). Stool frequency at discharge did not correlate with PHES ($P = 0.71$) or MOCA score ($P = 0.51$).

CONCLUSIONS: Traditional prognostic tools in cirrhosis, including MELD-Na and EHOFS, were superior to cognitive assessments, total daily lactulose dose, and stool frequency in predicting 30-day outcomes for those admitted with overt HE. Future studies should evaluate MELD-Na and the presence of EHOFS as determinants of discharge readiness or discharge destination in patients admitted with cirrhosis and HE.

P: 15 Junior Investigator | Oral Presentation

Functional MRI: Evidence of a Treatment Effect of LOLA

Yasmin Pasha, MBBS, MRCP$^1$, Sebastian A. Atzori, MBBS$^1$, Julie A. Fitzpatrick$^2$, Nicola A. Cook$^3$, Adrian Scenarbi, PhD$^2$, Robert Leech, PhD$^3$, Mark J. W. Mepham, MBBS, MRCP, PhD$^3$.

$^1$Imperial College London, UK; $^2$RMIT University, Melbourne, Australia; $^3$Kings College, London, UK.

BACKGROUND: Minimal hepatic encephalopathy (MHE) is associated with structural and functional connectivity abnormalities in the brain which correlate with cognitive dysfunction. The default mode network (DMN) comprises functionally interconnected brain regions responsible for attention and may explain cognitive deficits in MHE. While L-ornithine L-aspartate (LOLA) 6g three times per day with placebo on 34 individuals with MHE (defined by PHES) for 12 weeks. Fourteen subjects received LOLA. 20 patients received placebo for the study duration. Subjects underwent functional MRI (fMRI) of the brain while performing motor and cognitive tasks and resting state studies at baseline (before starting on LOLA) or placebo, and at 12 weeks. The motor and cognitive task data in the LOLA arm (vs placebo) were analysed by comparing the sum total of fMRI activation at baseline with data collected at 12 weeks. For resting state data, the level of functional connectivity within a network was compared between baseline and 12 weeks for LOLA against placebo.

METHODS: We performed a double-blind, randomised trial comparing the impact of oral L-ornithine L-aspartate (LOLA) 6g three times per day with placebo on 34 individuals with MHE. We performed a double-blind, randomised trial comparing the impact of oral L-ornithine L-aspartate (LOLA) 6g three times per day with placebo on 34 individuals with MHE. We performed a double-blind, randomised trial comparing the impact of oral L-ornithine L-aspartate (LOLA) 6g three times per day with placebo on 34 individuals with MHE. We performed a double-blind, randomised trial comparing the impact of oral L-ornithine L-aspartate (LOLA) 6g three times per day with placebo on 34 individuals with MHE.
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RESULTS: A total of 187 patients with a diagnosis of cirrhosis were approached for recruitment in the trial. 98 patients were excluded, whilst 17 patients declined. A total of 72 participants were enrolled in the trial. 37 participants had MHE whilst 35 participants did not have MHE. 16 participants had sarcopenia and MHE, whilst 14 participants had sarcopenia with no MHE. Over the average two year follow up, participants who did not have MHE and were not sarcopenic did not develop overt hepatic encephalopathy. Whilst participants with MHE and Sarcopenia had a significantly higher risk of developing OHE and mortality as can be seen in Figure 1 P value < 0.05. The Stroop test had the highest sensitivity 79% [CI 47%-86%] whilst the CFF had the highest specificity 78% [CI 66%-87%] P value 0.005. In the subset analysis of patients who underwent a DEXA scan to assess lean body mass, this was not able to predict the risk of developing OHE.

CONCLUSIONS: MHE and Sarcopenia assessment can be easily completed as a bedside clinical test to predict the long-term risk of mortality and the development of overt hepatic encephalopathy.

P: 18 Junior Investigator

Impaired Cerebral Oxygenation, but Preserved Cerebrovascular reactivity, in an Animal Model of Hepatic Encephalopathy

Anna Hadjihambi, PhD1,2, Patrick S. Hofold, PhD3, Abeba Haltection2, Nathan Davies, PhD2, Alexander V. Gourine, PhD3, Rajiv Jalan, PhD, MD2.
1Universite de Lausanne, Department of Physiology, Lausanne, Switzerland; 2UCL Institute for Liver and Digestive Health, Division of Medicine, University College London Medical School, Royal Free Hospital, London, UK; 3Centre for Cardiovascular and Metabolic Neurosciences, Neurosciences, Physiology and Pharmacology, University College London, London, UK.

BACKGROUND: We have recently obtained evidence of energy deficiency, in the form of impaired lactate release, in the brains of cirrhotic animals with hepatic encephalopathy (HE). Previous reports of cerebral hyperperfusion in patients with HE indicated that cerebral oxygen supply could also be compromised (Dam et al., 2013). Decreased lactate and reduced oxygen supply may lead to CNS energy deficiency and have important neurological consequences, particularly in patients with advanced cirrhosis. In this study we assessed cerebral tissue oxygen tension and CO2 cerebrovascular reactivity in an animal model of HE.

METHODS: HE was induced by bile duct ligation (BDL) and after 4 weeks rats were anesthetized with α-chloralose (100 mg·kg−1), instrumented for arterial blood pressure recording and artificially ventilated. 7 BDL and 6 sham-operated animals were treated daily for one week with an ammonia lowering treatment, ornithine phenylacetate (OP) in order to investigate the role of ammonia on brain oxygenation. Blood gas tensions and pH were maintained within physiological ranges in all animal groups. Cerebral tissue PO2 was monitored by fluorescence method (OxyLite™). After a small craniotomy, optical sensors were placed in the somatosensory cortex and sealed. PO2 at baseline and in response to systemic hypercapnia (10% CO2, 5 min) was recorded.

RESULTS: BDL resulted in high plasma ammonia concentrations which was lowered with OP treatment. At similar levels of blood PO2 and PCO2, BDL rats had a significantly lower brain PO2 (15.3 ± 2 mm Hg, n = 10) compared to sham controls (26 ± 2 mm Hg, n = 6; P = 0.001). BDL rats treated with OP showed a significant improvement in cerebral PO2 (22 ± 1 mm Hg, n = 6, P = 1), increasing the oxygen tension to levels similar to that recorded in OP treated sham rats (27 ± 2 mm Hg, n = 7), when blood PO2 and PCO2 were constant. Systemic hypercapnia resulted in similar increases in cerebral PO2 in BDL and sham-operated animals (ΔPO2 21 ± 2 vs. 24 ± 2 mm Hg, P = 0.6). Additionally, under anesthesia, the mean systemic arterial blood pressure was found to be significantly lower in BDL animals (60 ± 3 vs. 84 ± 8 mm Hg, P = 0.04). Cerebral oxygenation did not recover when the blood pressure was normalised via infusion of phenylephrine, but it significantly improved with infusion of acetazolamide which increases cerebral blood flow.

CONCLUSIONS: In the BDL model of HE, cerebral tissue oxygen tension is compromised but cerebrovascular reactivity to CO2 appears to be preserved. The cause of the low basal PO2 remains unknown however; high ammonia concentrations and hyperperfusion could be contributing factors.

P: 19 Junior Investigator

Predictive Value of Induced Hyperammonaemia and Neuropsychiatric Profiling in Relation to the Occurrence of Post-tips Hepatic Encephalopathy (HE)

Marco Sentzolo, MD, PhD1, Lisa Zarrantinello, PhD1, Chiara Formentin, MD2, Costanza Orlando, MD2, Raffaello Beltrame, MD2, Anna Vuerich, MD2, Paolo Angeli, MD2, Patricia Burr, MD2, Sara Montagnese, MD, PhD1.
1University of Padova, Padova, Italy.

BACKGROUND: Hepatic encephalopathy (HE) occurs in 20%–50% of patients after transjugular intrahepatic portosystemic shunt (TIPS). While indices such as an older age, a history of overt HE and severe liver failure have been associated with post-TIPS HE, it remains difficult to identify patients at risk. The aim of this study was to evaluate the predictive value of a large set of clinical, laboratory and neuropsychiatric parameters, both at baseline and after the induction of hyperammonaemia, in relation to the development of post-TIPS HE for 12 months in a group of well-characterised TIPS candidates.

METHODS: 18 TIPS candidates (38 ± 8 yrs, MELD 11 ± 3, 13 refractory ascites, 1 hydrothorax, 4 uncontrolled bleeding) underwent neurophysiological [Electroencephalography (EEG)], neuro-psychological [Psychometric Hepatic Encephalopathy Score (PHES) and Scan test], capillary ammonia and subjective sleepiness assessment both at baseline and after the induction of hyperammonaemia by an oral amino acid challenge (AAC). 6 months after TIPS, 3 patients had had an HE-related hospitalization. Compared to their counterparts who had not, they showed significantly lower, pre-AAC fasting ammonia concentrations (96 ± 93 vs. 225 ± 129 μmol/dL, P = 0.038) and subjective sleepiness (F(4, 52) = 2.7213, P = 0.038) and subjective sleepiness (F(4, 52) = 5.4002, P = 0.001). Six months after TIPS, 3 patients had had an HE-related hospitalization. Compared to their counterparts who had not, they showed significantly lower, pre-AAC fasting ammonia concentrations (96 ± 93 vs. 225 ± 129 μmol/dL, P = 0.038). They also showed worse PHES and Scan performance at baseline, as well as worse Scan performance and slower EEG post-AAC. Twelve months post-TIPS, 5 patients had had an HE-related hospitalization, and comparisons between the two groups confirmed the findings at 6 months.

CONCLUSIONS: TIPS candidates, who are by definition at low risk of HE, seem to encompass two different populations: i) those who are well and have not had overt HE because their ammonia levels are near-normal, ii) those who are well and have not had overt HE despite hyperammonaemia, either because of habituation or a personal inclination not to exhibit the HE phenotype. Fasting ammonia may be a promising and easily obtained parameter for future validation and inclusion in models for the prediction of post-TIPS HE.
P: 20 Junior Investigator

EID-UL-AZHA (Muslim’s Festival of Sacrifice): Increased Frequency of Spontaneous Encephalopathy, Whether This Is Secondary to Consumption of High Protein Diet?

Rehmatullah Bhatti1
1Asian Institute of Medical Sciences (AIMS), Hyderabad, Pakistan.

BACKGROUND: Protein restricted diet once was the cornerstone of the management of Hepatic encephalopathy, latter no evidence showed significant benefit. However sub group of cirrhotics are intolerant to dietary proteins, especially animal proteins. We compared precipitants of encephalopathy and effect of animal proteins consumption during the festival of EID-UL-AZHA

METHODS: A retrospective comparative cross sectional study was conducted and analyzed hospital data of AIMS (Asian Institute of medical sciences, Hyderabad, Pak.). Patients of cirrhosis were enrolled with clinically diagnosed hepatic encephalopathy in two groups. Group A was categories with patients admitted 15 days before EID-UL-AZHA and Group B includes patients admitted on EID UL AZHA day and up to 15 days afterward. Patients with neurological deficit and suspected CNS infection were excluded. Patient of both groups were assessed to compare precipitant factors for encephalopathy that includes infections, electrolytes imbalance, upper GI bleed, constipation, AKI and drugs. Dietary history regarding normal protein diet and high protein diet (animal proteins > 1.5 g/kg/day) were also collected and compared in both groups.

RESULTS: Out of 57 screened patients, 92 were presented with encephalopathy. All patients were Muslims with mean age 54.58 ± 11 yrs. Majority 66.3% were males and 33.7% were females. Precipitating factors were identified in 70.2% and 62.9% in group A and B respectively. Precipitating factor remain unidentified in 29.8% patients in group A and 37.1% in group B. 98.2% patients in group A were taking usual diet, only 1.8% consumed high (animal) protein diet. while in group B 51.4% patients were on usual diet and 48.6% consumed high (animal) protein diet. A comparable rise in frequency of spontaneous hepatic encephalopathy (without an identified precipitating factors) was observed in group B and 76.9% patients out of them were consuming high (animal) protein diet.

CONCLUSIONS: A compatible rise in frequency spontaneous hepatic encephalopathy was observed during and/or post event of EID-UL-AZHA, majority had consumed high (animal) proteins. Consumption of meat (animal proteins) could be the possible contributing factor. Generally protein restriction is not recommended in cirrhosis but, still in sub group of cirrhotics who are protein intolerant, protein restriction or substitution of source of protein is inevitable. A further multicenter study is necessary at a larger scale to find out characteristic of patients who are intolerant to animal proteins.

P: 21 Junior Investigator

Changes in Cerebral Hemodynamic Parameters in Patients With Acute Liver Failure

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1National Institute of Medical Sciences and Nutrition Salvador Zubirán, Mexico City, Mexico.

BACKGROUND: Acute liver failure (ALF) is a clinical syndrome that results from severe and sudden loss of liver function in a patient without preceding liver disease. High intracranial pressure (ICP) leads to loss of cerebral perfusion, brain herniation and irreversible brain damage in patients with acute liver failure (ALF).1 Transcranial Doppler ultrasound (TCD) is a non-invasive device that can continuously measure the speed of cerebral blood flow, producing a velocity-time waveform that indirectly monitors changes in cerebral hemodynamics, making it very useful in patients with ALF.3,4 Objectives. Compare the cerebral hemodynamic parameters in patients with ALF using TCD before and after liver transplantation.

METHODS: Retrospective and descriptive study. We searched patients with ALF during the period 2014–2018, who underwent TDC. For the descriptive analysis, medians of the quantitative variables and percentages of the qualitative variables were used. The comparison of medians was made using t test. Values were expressed as median and interquartile range, analyzed with Mann-Whitney U test. A value of P < 0.05 was considered statistically significant.

RESULTS: We studied 10 patients (9 women, 1 man), with a median age of 29 years. The etiology of the liver failure was the following: 50% for autoimmune hepatitis, 20% undetermined, 10% hepatitis A and 10% pharmacological. We identified 4 deaths, 1 of which was during the post-transplant period. The following parameters were measured: systolic peak velocity (SPV), final diastolic velocity (FDV), medium velocity of the middle brain artery (VMD), resistance index (RI) and pulsatility index (PI). There was changes in RI and PI, before transplant TDC: RI 0.69 (0.58–0.71) PI 1.29 (0.96–1.37) and after transplant TDC: RI 0.51 (0.0-0.58), PI 0.78 (0.55–0.95) P value = 0.005 and 0.005 respectively.

CONCLUSIONS: There was a significant decrease in the rates of pulsatility and resistance after liver transplantation.

REFERENCES

P: 22 Junior Investigator

Deficit of Short Working Memory in Rat With Thioacetamide-Induced Progressive Acute Hepatic Encephalopathy Involving Serotonin Innervation and Astroglia Dysfunctions

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1Cadi Ayyad University, Marrakech, Morocco; 2Chouaib Doukkali University, El Jadida, Morocco.

BACKGROUND: Hepatic encephalopathy (HE) is defined as the whole neurological and neuropsychiatric disorders arising from both acute and chronic liver failures. Patients with chronic HE exhibited an impairment of learning and memory abilities as well as attention. The aim of the present study is to evaluate the progression of short working memory alteration in acute HE and to delineate the glial and the neuronal alterations which may underlie such cognitive impairment.

METHODS: The study was performed in Sprague-Dawley rats with acute liver failure induced by thioacetamide (TAA) at a dose of 300 mg/kg i.p. Different stages of acute HE was defined as: 12, 24h and 36 h following administration of TAA. Working memory was assessed by the T-Maze test via the percentage of alternation behavior, as well, an immunohistochemical analysis of GFAP in the hippocampus and serotonin (5-HT) within the dorsal Raphe nucleus (DRN).

RESULTS: Our data showed a progressive loss of the alternation behavior, which was accompanied by a time dependent and region-specific changes of GFAP-immunoreactive astrocytes within the hippocampus together with a reduced 5-HT immunoreactivity within the DRN.

CONCLUSIONS: Our data revealed for the first time, a progressive loss of short memory function in acute HE, resulting from acute liver dysfunction which may involve a possible gliopathy as well as a 5-HTergic dysfunctions.

P: 23 Junior Investigator

Prospective Study on Mortality of Acute on Chronic Liver Failure and Its Predictors

Rehmatullah Bhatti1
1Asian Institute of Medical Sciences (AIMS), Hyderabad, Pakistan.
CONCLUSIONS: Acute-on-chronic liver failure (ACLF) is an acute deterioration of liver function and a high mortality. We aimed to determine hospital, 28 days and 12 weeks mortality of ACLF, its predictors and precipitating factors.

METHODS: We conducted a prospective descriptive study at AIMS (Asian institute of medical sciences, Hyderabad, Pak.) from January 2018 to December 2018. We enrolled patients of ACLF as defined by Asian Pacific Association for the Study of Liver (APASL, 2014) and collected data to determine cause, precipitating acute insult, organ failure, ACLF grade MELD, and CTP scores.

RESULTS: Total patients were 117 with mean age of 40.9 ± 13.9 years (range 12–85). Majority were males 86 (73.5%) and 31 (26.5%) were females. Majority of patients 55 (47%) were Hepatitis B Virus (HBV) positive, among them 24 (43.6%) were with HDV co-infection. The most common precipitating acute insult was SEPSIS 65 (55.6). Others were drug induce liver injury (DILI) 8 (6.8%), HEV acute hepatitis 7 (5.9), HDV superinfection 5 (4.3%), HBV flair 4 (3.4%), alcohol binge drinking 4 (3.4%), surgery 2 (1.7%), acute PVT 2 (1.7%), Upper GI Bleed 1 (0.9%) and 20 (17.1%) were unknown. Hospital mortality was 49 (41.9%), 28 days 71 (60.7%) and 12 weeks mortality was 103 (88.0%). Organ failure (P < 0.001), ACLF grade (P < 0.001), encephalopathy (P = 0.001), MELD (P = 0.01) and AKI (P = 0.02) were found to be predictors of mortality.

CONCLUSIONS: Acute-on-chronic liver failure (ACLF) is an acute deterioration of liver function superimposed on Chronic Liver Disease with a high mortality. In our study HBV infection was the commonest cause of CLD, and sepsis was the commonest acute insult. We found high hospital, 28 days and 12 weeks mortality. Organ failure, ACLF grade, encephalopathy, MELD score and AKI were found to be predictors of mortality of ACLF.

P: 24 Junior Investigator

Comparative Morphological Analysis of Astroglia Reactivity in the Hippocampus of Rats With Acute and Chronic Hepatic Encephalopathy

El Hiba Omar, PhD1,2, El Khiat Abdelaati2, Aitihya Mohamed2, Gamrani Halima2.

1Department of Translational and Precision Medicine, Silvia Nardelli1, Barbara Lattanzi1, Manuela Menti1, Stefania Giusi1, Lorenzo Ridol1, Oliviero Riggio2.

BACKGROUND: Hepatic encephalopathy (HE) is a serious complication of advanced liver failure which represents the whole neuropsychiatric abnormalities resulting from liver disease ranging from abnormal behavior to coma. Impairment of cognitive function is well described in HE patients, while mechanisms of memory impairment in HE patients is still not fully understood, however, it may involve a possible gliopathy as well as neuropathy in various brain areas, including the hippocampus known as the main brain structure associated to the memorization process. The aim of the present investigation is to assess astroglia reaction of the hippocampus, in a comparative approach between acute and chronic HE in rat.

METHODS: Sprague-Dawley rats were divided into 3 groups controls; rats were treated with saline solution (NaCl 0.9 % i.p) during 3 days, chronic HE group: rats were subjected to Bile Duct Ligation and acute HE group: rats were subjected to 3 i p injections of thioacetamide (TAA) 300 mg/kg BW. Astroglia was assessed through an immunohistochemical analysis using anti-GFAP antibody on frontal sections of the hippocampus following.

RESULTS: Our data showed in the TAA rats (3 days following 3 TAA injections) compared to controls, a significant increase of GFAP immunoreactivity within the whole hippocampal areas, while astrocytic processes length and ramification were reduced, the GFAP immunoreactive area was increased. In contrast to BDL rats, at the cirrhotic stage (4 weeks after surgery), showing the opposite tendency with a drop of the astrocytic GFAP-immunoreactive area as well as the astrocytic length and ramification levels.

CONCLUSIONS: The present finding sustains a differential astroglial reactivity within the hippocampus of acute and chronic HE rats. Astrocytic morpholody changes depends to the severity of liver failure and seems to HE type-dependent, while those astroglial changes lead to a severe gliopathy which may be behind the disturbed cognitive function, especially memory seen in patients with chronic as well as acute HE.

P: 25 Junior Investigator

Muscle Alterations Are Associated With Minimal and Overt Hepatic Encephalopathy in Patients With Liver Cirrhosis

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1Department of Translational and Precision Medicine, “Sapienza” University of Rome, Rome, Italy.

BACKGROUND: Muscle alterations (myosteatosis and sarcopenia) are frequent in cirrhosis and related to some complications included overt hepatic encephalopathy. The aim of our study was to investigate the relationship between muscle alterations and minimal hepatic encephalopathy (MHE) and their role on the risk of overt HE.

METHODS: 64 cirrhotics were submitted to Psychometric Hepatic Encephalopathy Score (PHEs) and to Animal Naming Test (ANT) to detect MHE. CT scan was used to analyse the skeletal muscle index (SMI) and attenuation. The incidence of the first episode of HE, taking into account the competing risk nature of the data, was estimated.

RESULTS: Myosteatosis was observed in 24 patients (37.5%), sarcopenia in 37 (58%) and MHE in 32 (50%). Both myosteatosis (62.5 vs 12.5%; P < 0.001) and sarcopenia (84 vs 31%; P < 0.001) were more frequent in patients with MHE. The variables independently associated to the presence of MHE were: sarcopenia, previous overt HE and myosteatosis. Thirty-one (48%) patients developed overt HE during 16.1 ± 13 months; myosteatosis was detected in 68% and sarcopenia in 84% of them. Sarcopenia and myosteatosis were also independently associated to the development of overt HE. Venous ammonia was significantly higher in sarcopenic patients (62.6 ± 17.7 vs 41.4 ± 16.1 μg/dl; P < 0.001) and in myosteatotic patients (65.2 ± 19.2 vs 46.7 ± 17.1 μg/dl, P < 0.001) and inversely correlated to both parameters. Survival was significantly lower in malnourished patients compared with patients without myosteatosis or sarcopenia (P < 0.001).

CONCLUSIONS: Myosteatosis and sarcopenia, probably by reducing the handling of ammonia in the muscle, are independently associated to MHE and to the risk of overt HE in cirrhosis. In malnourished patients, the amelioration of nutritional status may be a possible goal to decrease both the prevalence of MHE and the incidence of overt HE.
The Modification of Quantity and Quality of Muscle Mass Improves the Cognitive Impairment After TIPS

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BACKGROUND: Hepatic encephalopathy (HE) is the major complication of transjugular intrahepatic portosystemic shunt (TIPS). In cirrhotic patients, a correlation between sarcopenia and hepatic encephalopathy has been suggested.

AIM: to evaluate the evolution of the skeletal muscle quantity and quality at CT scan and of the patients’ cognitive impairment (both overt and minimal HE) before and after TIPS.

PATIENTS AND METHODS: 27 cirrhotic patients submitted to TIPS were studied. The modification of Skeletal Muscle Index (SMI), muscle attenuation, HE and plasma ammonia were evaluated before and after a mean follow-up of 9.8 ± 4 months after TIPS.

RESULTS: During the follow-up, the mean SMI and muscle attenuation increased significantly, although not uniformly in all patients. PHES (Psychometric Hepatic Encephalopathy Score) and ammonia improved significantly in the patients with amelioration in SMI >10% (n = 16) and not in those without (n = 11) (PHES: −1.6 ± 2 vs −4.8 ± 2.1; P = 0.0005; ammonia: 48.5 ± 28.7 vs 96 ± 31.5 μg/dl; P = 0.0004). Moreover, the prevalence of minimal HE (12.5% vs 73%, P = 0.001) as well as the number of episodes of overt HE during the follow-up were significantly reduced in the patients with improved SMI. MELD remained stable or worsened after TIPS and was not significantly different between the groups with or without SMI improvement.

CONCLUSION: The amelioration of muscle wasting and HE independent of liver function observed after TIPS supports the causal relationship between muscle wasting and HE.

Evaluation of Neurocognitive Function in Patients With Compensated and Decompensated Cirrhosis

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BACKGROUND: Cirrhosis is the end result of chronic liver damage caused by multiple entities. Up to 20%–60% of patients with cirrhosis are affected by a peculiar type of mild cognitive impairment related to selective attention and executive functions, visuomotor capacity, psychomotor speed, inhibition of response and selection of response that can only be detected by psychometry.

METHODS: An observational, cross-sectional, analytical study was carried out. A total sample of 126 patients was established, 64 patients for each group. The NeuroPsi instrument was applied to determine the domains (Orientation, Attention, Visual Episodic Memory, Verbal Episodic Memory, Language, Reading Writing, Executive Conceptual Functions and Executive Motor Functions); With the transcranial Doppler, the hemodynamic parameters such as velocity, pulsatility index, resistance index, as well as the aplea index to assess cerebral vaso-reactivity were evaluated.

RESULTS: Up to now, 55 patients were studied, two groups were established: compensated cirrhotic 18 and decompensated, the gender ratio was 38% men and 62% women, with a median of 60 years. Compensated cirrhotics showed a higher percentage of patients without hepatic encephalopathy (HE) 65% vs 27.1%. The mean Child Pugh score was 7.47 ± 1.82. MELD Na mean for total patients was 12.98 ± 5.04. Of the masters, Reading Writing with 4.50 ± 0.89 for compensated and 4.85 ± 0.50 in decompensated (P = 0.07), in the domain of Executive Motor Functions with 5.69 ± 1.8 for compensated and 6.24 ± 0.89 compensated (P = 0.16).

CONCLUSIONS: This study could not find significant differences in the averages obtained in the 8 cognitive domains explored, between the compensated and decompensated cirrhotic patients, nor correlation between the Child Pugh score and the performance in the cognitive tests; It is considered relevant to increase the sample of this study to have more solid evidence. In the cerebral hemodynamic parameters assessed by transcranial Doppler, there were no significant differences between compensated and decompensated patients.

Prevalence of cognitive impairment (minimal and overt HE) in the patients with or without improvement of SMI >10% at the end of follow-up

<table>
<thead>
<tr>
<th></th>
<th>SMI improvement&lt;10% (n=11)</th>
<th>SMI improvement&gt;10% (n=16)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal HE</td>
<td></td>
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<tr>
<td>(PHES&lt;4) (yes/no)</td>
<td>8/3 (72.7%)/(27.3%)</td>
<td>2/14 (12.5%)/(87.5%)</td>
<td>0.001</td>
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<tr>
<td>PHES score</td>
<td>−4.8±2.1</td>
<td>−1.6 ±2</td>
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<td>Overt HE (yes/no)</td>
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<td>OHE in the 3 months</td>
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<td>(N of episodes/pt)</td>
<td>0.9±1.04</td>
<td>0.6±0.5</td>
<td>0.3</td>
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<td>OHE in the following months</td>
<td>1.4±1.4</td>
<td>0.06±0.3</td>
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<tr>
<td>(N of episodes/pt)</td>
<td></td>
<td></td>
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<tr>
<td>Venous plasma Ammonia (μg/dl)</td>
<td>96±31.5</td>
<td>48.5±28.7</td>
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<td>Follow up (months)</td>
<td>11.9±6.2</td>
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</table>

Mean ± SD

[26]
BACKGROUND: Falls are common and associated with significant morbidity for persons with cirrhosis and hepatic encephalopathy. In order to intervene with targeted rehabilitation efforts to reduce and prevent falls, a better understanding of mechanisms underlying balance deficits is needed. To this end, unipedal stance time (UST) is a useful measure of balance as it requires the integration of sensory, neurocognitive, and muscular factors.

METHODS: We prospectively enrolled 119 outpatients with cirrhosis and history of hepatic encephalopathy from the hepatology clinic at Michigan Medicine. The primary outcome was UST. Each patient was evaluated for demographic and clinical factors (Child-Turcotte Pugh (CTP) class, MELD score), physical function (grip-strength and hip-strength using lateral plank time), neurocognitive factors [Numbers Connection Test (NCT) A and B], recognition reaction time (ms), self-efficacy, perceived ability to perform different tasks without falling, was also assessed. We evaluated bivariate Pearson correlations and developed a linear regression model to identify significant contributors to balance impairment.

RESULTS: Participants were 50% female, aged 62.9 ± 7.3 years, 80% CTP A, 18% CTP B, 2% CTP C, with MELD 11 ± 5. The mean UST was 12.7 ± 9.9 seconds (median = 9.42 seconds, IQR = 19.26). In bivariate analyses, UST was most highly correlated with lateral plank time (r = 0.61), followed by recognition reaction time accuracy [total, percent correct in lights on, and in lights off conditions (r = 0.47, r = 0.36, r = 0.33)] and NCT A and B tests (each r = 0.27). In multivariable regression, 54% of variance in UST was explained by significant factors of plank time, recognition reaction time accuracy, falls self-efficacy, age, and CTP. Based on standardized beta coefficients, plank time, CTP of B or C, and recognition reaction time accuracy were the strongest predictors. For every second increase of plank time, UST increases by 0.26 seconds on average. If classified by CTP of B or C, UST decreases by 5.8 seconds. For each additional percent correct on reaction time accuracy, UST increases by 0.12 seconds.

CONCLUSIONS: The strongest factors associated with diminished balance, as indicated by UST, in this high fall-risk cirrhosis population are hip strength, clinical disease severity, and neurocognitive capacity. These findings support a rehabilitation approach that targets strengthening as well as neurocognitive training to address balance impairment.

P: 30 Junior Investigator

Relation Between Mortality and Psychometric Test Results in Patients Awaiting Liver Transplantation

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BACKGROUND: Hepatic encephalopathy is a common severe complication of liver cirrhosis that, however, so far is not considered as an indication for organ allocation to patients on the transplant waiting list. Multiple psychometric tests have been developed to detect hepatic encephalopathy. However, there is only rare data about the predictive value of psychometric test results regarding mortality in patients with liver cirrhosis. This retrospective analysis of prospective data determined the predictive value of the Inhibitory Control Test (ICT), the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), and the Behavioral Inhibition System/Inhibitory Control Test (BIS/BIS-IT). The primary outcome was mortality in patients with liver cirrhosis. This retrospective analysis of prospective data determined significant predictors of mortality in these patients.

METHODS: A total of 119 patients with liver cirrhosis were included in this study. The average age of the patients was 62.9 ± 7.3 years, 80% CTP A, 18% CTP B, 2% CTP C, and MELD 11 ± 5. The mean UST was 12.7 ± 9.9 seconds (median = 9.42 seconds, IQR = 19.26). In bivariate analyses, UST was most highly correlated with lateral plank time (r = 0.61), followed by recognition reaction time accuracy [total, percent correct in lights on, and in lights off conditions (r = 0.47, r = 0.36, r = 0.33)] and NCT A and B tests (each r = 0.27). In multivariable regression, 54% of variance in UST was explained by significant factors of plank time, recognition reaction time accuracy, falls self-efficacy, age, and CTP. Based on standardized beta coefficients, plank time, CTP of B or C, and recognition reaction time accuracy were the strongest predictors. For every second increase of plank time, UST increases by 0.26 seconds on average. If classified by CTP of B or C, UST decreases by 5.8 seconds. For each additional percent correct on reaction time accuracy, UST increases by 0.12 seconds.

CONCLUSIONS: The strongest factors associated with diminished balance, as indicated by UST, in this high fall-risk cirrhosis population are hip strength, clinical disease severity, and neurocognitive capacity. These findings support a rehabilitation approach that targets strengthening as well as neurocognitive training to address balance impairment.

P: 29 Junior Investigator

Contributors to Balance Impairment in Adults With Cirrhosis and Hepatic Encephalopathy

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Accuracy of DKI/DTI parameters for diagnosis of minimal hepatic encephalopathy among cirrhotic patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AUROC</th>
<th>Cut-off</th>
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of Neuropsychological Status (RBANS), the Portosystemic Encephalopathy Syndrome-Test and the Critical Flicker Frequency (CFF) assessment in regard to mortality in patients on the waiting list for liver transplantation.

METHODS: 143 patients awaiting liver transplantation were included. They underwent a test battery including the Inhibitory Control Test, the Repeatable Battery for the Assessment of Neuropsychological Status, the Portosystemic Encephalopathy Syndrome-Test which provides the psychometric hepatic encephalopathy score (PHES) and the Critical Flicker Frequency assessment at study inclusion. The PHES was available for all patients \( n = 143 \), the RBANS scores for \( n = 115 \), the ICT results for \( n = 99 \), and the CFF results for \( n = 136 \) patients. Basic characteristics (age, gender, underlying liver disease, accompanying diseases) and Model for End-stage Liver Disease (MELD)-Score at the time of study inclusion were documented. Follow-up was done for 5 years. Patients who either received a liver transplantation or dropped out of the study during the observation period were censored. The median survival following cohort entry of those who did and did not develop HE was 5.78 and 3.4 years, respectively \( (P < 0.001) \). Multivariate analysis identified decreased survival with older age \( (HR 1.02, CI 1.02–1.03) \), male sex \( (HR 1.21, CI 1.19–1.24) \), ESRD \( (HR 1.08, CI 1.01–1.14) \), and increasing Charlson Comorbidity Index \( (HR 1.27, CI 1.17–1.48) \). Cirrhosis etiologies of HCV and alcohol were associated with improved survival \( (HR 0.87 CI: 0.85–0.90) \), while NAFLD was linked to increased mortality after HE \( (HR 0.88, CI: 0.85–0.90) \) and HR 0.88, CI 0.79–0.85, respectively) \( (P = 0.029) \). Factors that were inversely associated with hospital utilization were Rifaximin use \( (HR 0.40, CI: 0.39–0.42) \) and gastroenterology consultation \( (HR 0.73, CI: 0.67–0.80) \). Rifaximin use was associated with decreased hospital-days \( (IRR 0.35, CI: 0.33–0.37) \) and fewer 30-day readmissions \( (HR 0.18, CI: 0.08–0.40) \), while gastroenterology consultation was associated only with a decreased risk of 30-day readmissions \( (HR 0.71: CI: 0.57–0.88) \) but not overall hospitalizations.

DISCUSSION: The outcomes after HE in this contemporary Medicare-insured population are poor. The development of HE increases hospitalization utilization and worsens survival with few, potentially modifiable, targets for prospective study intervention.

P: 31 Junior Investigator

The Natural History of Cirrhosis After the Development of Hepatic Encephalopathy
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1University of Michigan, Ann Arbor, MI, USA.

BACKGROUND: Hepatic encephalopathy (HE) is a watershed moment in the natural history of cirrhosis portending decreased quality of life and worsening prognosis. Recent strides in the management of HE have been made to decrease symptom burden and readmissions. The impact of these interventions requires a contemporary re-examination of the natural history of HE and its clinical implications.

METHODS: We examined data from a 20% random sample of US Medicare enrollees with cirrhosis and continuous Part D prescription coverage from 2008–2014. Those with a diagnosis of HE prior to or within 3 months after the diagnosis of cirrhosis was made were excluded. Incident HE was defined by ICD-9 code 572.2 and/or the initiation of a prescription for an HE-specific treatment (Neomycin, Lactulose, or Rifaximin). Outcomes included transplant-free survival and hospital-days or 30-day readmissions per person-year. Multivariate analysis was performed for survival (hazard ratios, HR, Cox regression) and hospital utilization (incidence rate ratios, IRR, negative binomial regression).

RESULTS: Among 186,160 Medicare-enrollees (median age 65 years) with cirrhosis, 49,164 experienced HE (26.4%). The median survival following cohort entry of those who did and did not develop HE was 5.78 and 3.4 years, respectively \( (P < 0.001) \). Multivariate analysis identified decreased survival with older age \( (HR 1.02, CI 1.02–1.03) \), male sex \( (HR 1.21, CI 1.19–1.24) \), ESRD \( (HR 1.08, CI 1.01–1.14) \), and increasing Charlson Comorbidity Index \( (HR 1.27, CI 1.17–1.48) \). Cirrhosis etiologies of HCV and alcohol were associated with improved survival \( (HR 0.87 CI: 0.85–0.90) \), while NAFLD was linked to increased mortality after HE \( (HR 0.88, CI 0.79–0.85, respectively) \) while NAFLD was linked to increased mortality after HE \( (HR 1.07, CI 1.02–1.12) \). Hospital-days per person year were 11.8 in patients with HE compared to 2.9 in those without \( (P < 0.001) \). Factors that were inversely associated with hospital utilization were Rifaximin use \( (HR 0.40, CI: 0.39–0.42) \) and gastroenterology consultation \( (HR 0.73, CI: 0.67–0.80) \). Rifaximin use was associated with decreased hospital-days \( (IRR 0.35, CI: 0.33–0.37) \) and fewer 30-day readmissions \( (HR 0.18, CI: 0.08–0.40) \), while gastroenterology consultation was associated only with a decreased risk of 30-day readmissions \( (HR 0.71, CI: 0.57–0.88) \) but not overall hospitalizations.

DISCUSSION: The outcomes after HE in this contemporary Medicare-insured population are poor. The development of HE increases hospitalization utilization and worsens survival with few, potentially modifiable, targets for prospective study intervention.

P: 32 Junior Investigator

Liver Transplant Is Associated With Improvement in Cognition, Tandem Gait and Risk of Falls
Chathur Acharya, MD1, Melanie B. White, RN1, Andrew Fagan, Richard K. Sterling, MD2, R. Todd Stravitz, MD3, Punnet Puri, MD4, Michael Fuchs, MD5, Yelimir Luketic, MD6,
score at 6 month [2 pts (Table 1). Post-LT analysis showed that pts with abnormal pre-LT TD had a worse PHES, SIP scores, falls and TD abnormalities post-LT more at 12 compared to 6 months in all inhibitors and mycophenolate at post-LT visits. There was a sustained improvement in scores were significant [OR 0.8 (0.68-1.09), P = 0.02]. On multivariable analysis with abnormal TD as dependent variable physical SIP score was [24 (12.5, 39) vs 13.8 (3.2, 20.5), P = 0.008]. On multivariable analysis with abnormal TD as dependent variable physical SIP score was significant [OR 1.05 (1.006-1.09), P = 0.02]. On multivariable analysis with CHE as dependent variable physical SIP score [OR 1.12 (1.06-1.25), P = 0.02] and PHES scores were significant [OR 0.8 (0.68-0.96), P = 0.02]. Post-LT 6/12 months: Six pts had ACR that was managed with standard treatment and all pts were controlled on calcinurine inhibitors and mycophenolate at post-LT visits. There was a sustained improvement in PHES, SIP scores, falls and TD abnormalities post-LT more at 12 compared to 6 months in all pts (Table 1). Post-LT analysis showed that pts with abnormal pre-LT TD had a worse PHES score at 6 months [−4 (−5, 0) vs −0.5 (−3, 1.25), P = 0.0064] and higher cognitive impairment on PHES [12 (40%) vs 3 (10%), P = 0.007] compared to those with normal pre-LT TD. CONCLUSIONS: After LT, there is a sustained improvement in CF, abnormal TG and HRQOL from 6 through 12 months, which is accompanied by a lower rate of falls.

METHODS: Hippocampus (n = 7), cerebellum (n = 8) and striatum (n = 4) of adult male Wistar rats were scanned longitudinally using in-vivo 1H-MRS (SPECIAL sequence-TE = 2.8 ms, quantification with LCModel) at 9.4T before (week 0) and after bile duct ligation (BDL-CHE model). Scans and blood tests were performed every two-weeks till week 8.

RESULTS: All BDL rats showed an increase in plasma bilirubin and blood ammonia validating the presence of CLD. Increase in brain Glutamine (Gln) was observed for all brain regions being the most pronounced in cerebellum (+134%-week 8) (Figure 1c). Furthermore, this increase showed a strong correlation with blood ammonia for all three brain regions (Figure 1b). The main brain organic osomolytes (Inositol, Taurine, Creatine and total-Choline) displayed a similar decreasing trend in concentration as a response to Gln increase (osmoregulation) for both hippocampus and cerebellum, always having a stronger change for cerebellum. Interestingly, despite the smallest Gln increase, striatum showed more pronounced decrease in concentration of osomolytes than hippocampus (Figure 1d,e). Also, trend towards a decrease in NAA and PE was observed uniquely for striatum (data not shown). A tendency of increase in Lactate was observed being the strongest for cerebellum (+84%-cerebellum, +8%-hippocampus, +5%-striatum) indicating a possible energy metabolism perturbation (Figure 1f). Brain regions displayed different antioxidant response with a decrease in Ascorbate being stronger in cerebellum (data not shown).

CONCLUSIONS: This is the first study showing in-vivo longitudinal analysis of neuro-metabolism in three different brain regions in a model of CHE. Hippocampus and cerebellum displayed similar trends in metabolite changes during the course of disease, while the changes were much more pronounced in cerebellum. Striatum showed differences in metabolic response when compared to the other brain regions. Clinical relevance of these findings remain to be determined. We conclude that different brain regions are differentially susceptible to the metabolic consequences of CLD, a field which warrants further study.

REFERENCES

P: 33 Junior Investigator

In Vivo Longitudinal 1H MRS Study of Hippocampal, Cerebral and Striatal Metabolic Changes in the Adult Brain Using an Animal Model of Chronic Hepatic Encephalopathy

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BACKGROUND: Chronic hepatic encephalopathy (CHE) is a severe complication of chronic liver disease (CLD) characterized by cognitive and motor deficits. The diseased liver fails to metabolize toxins from the blood (ammonium, bilirubin etc.) which accumulate in the blood and brain. There is evidence that ammonium uptake rates differ among the brain regions. Since the CHE patients present various symptoms with different severity, the susceptibility to CHE and the mechanisms causing the damage may depend on the brain region. The aim of this study was to investigate, for the first time, potential metabolic differences between hippocampus, cerebellum and striatum as key brain regions implicated in manifestation of CHE.

METHODS: Hippocampus (n = 7), cerebellum (n = 8) and striatum (n = 4) of adult male Wistar rats were scanned longitudinally using in-vivo 1H-MRS (SPECIAL sequence-TE = 2.8 ms, quantification with LCModel) at 9.4T before (week 0) and after bile duct ligation (BDL-CHE model).

RESULTS: All BDL rats showed an increase in plasma bilirubin and blood ammonia validating the presence of CLD. Increase in brain Glutamine (Gln) was observed for all brain regions being the most pronounced in cerebellum (+134%-week 8) (Figure 1c). Furthermore, this increase showed a strong correlation with blood ammonia for all three brain regions (Figure 1b). The main brain organic osomolytes (Inositol, Taurine, Creatine and total-Choline) displayed a similar decreasing trend in concentration as a response to Gln increase (osmoregulation) for both hippocampus and cerebellum, always having a stronger change for cerebellum. Interestingly, despite the smallest Gln increase, striatum showed more pronounced decrease in concentration of osomolytes than hippocampus (Figure 1d,e). Also, trend towards a decrease in NAA and PE was observed uniquely for striatum (data not shown). A tendency of increase in Lactate was observed being the strongest for cerebellum (+84%-cerebellum, +8%-hippocampus, +5%-striatum) indicating a possible energy metabolism perturbation (Figure 1f).Brain regions displayed different antioxidant response with a decrease in Ascorbate being stronger in cerebellum (data not shown).

CONCLUSIONS: This is the first study showing in-vivo longitudinal analysis of neuro-metabolism in three different brain regions in a model of CHE. Hippocampus and cerebellum displayed similar trends in metabolite changes during the course of disease, while the changes were much more pronounced in cerebellum. Striatum showed differences in metabolic response when compared to the other brain regions. Clinical relevance of these findings remain to be determined. We conclude that different brain regions are differentially susceptible to the metabolic consequences of CLD, a field which warrants further study.

REFERENCES
METHODS: Patients were identified at two institutions (AIIMS & UCL) as part of ongoing prospective studies of AD. Culture positive infections and severity of HE (classified by West Haven Criteria) were measured on the day of admission, and new culture positive infections were assessed for up to 28 days after admission. Organ failures were defined as CLIF-organ failure score. Cox-proportional hazard analysis was used to assess predictors of infection.

RESULTS: 759 cirrhotic patients with AD were included with a median age of 45 years, and varying degrees of HE; grade 0/1 (n = 452), grade 2–4 (n = 307). On day 0, Patients classified into 4 groups; no HE no infection (n = 359), overt HE no infection (n = 222), no HE with infection (n = 93), overt HE with infection (n = 85). OfS (Lever, Renal, Brain, Coagulation, Respiratory, and Circulatory) and ACLEF grades were measured on Day 0, with ACLEF grade 0, 1, 2, and 3 (n = 242, 99, 206, and 212 respectively). On univariate and multivariate analyses, oVert HE (with no baseline infection) was independently predictive of new infections (1.639 and 1.608; P = 0.006, and 0.016 respectively). Furthermore, age and circulatory failure were also independent risk factors for infection (P = 0.001, and 0.017 respectively). Overall, HE was higher in non-survivors (n = 191) compared to survivors (n = 116).

CONCLUSIONS: The results of this study show for the first time that, in AD patients, overt HE not only associated with higher mortality but is also an independent risk factor for infection. We also showed that Age and Circulatory failure are independent risk factors for infections. That would make them, after further studies, an indication for prophylactic antibiotics.

P: 36
Junior Investigator

Antibiotic Rifaximin for Treatment of Chronic Liver Disease-Induced HE: A Longitudinal In Vivo 1H-MRS Study of Brain Metabolism on BDL Rats
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BACKGROUND: Rifaximin is a commonly-used antibiotic to treat hepatic encephalopathy (HE), a complex neuropsychiatric syndrome caused by hepatic dysfunction. Rifaximin aims at reducing the production of gut ammonia, an important toxin in HE pathogenesis. In a previous study using bile duct ligated (BDL) rats, we showed that rifaximin at the recommended human dose may help reduce brain Gln levels in early stages of HE.1 These findings raised the question of the efficacy of the dose used at later stages. Therefore we hypothesized that the effect of rifaximin on neurometabolic profile may be dose-related. In this study, the effects of a dose 6.2x that recommended in humans 2 were assessed in vivo and longitudinally in BDL rats. They were compared with non-treated rats (n = 17) and human-dose treated rats (15.7 mg/kg/day, n = 12).3

METHODS: Plasma measurements of NH4+, bilirubin and 1H-MRS scans were performed on adult Wistar rats (n = 8) before BDL (‘week 0’) and at weeks 2, 4, 6, 8 post-BDL. Rifaximin was administered twice daily (6x-human-dose = 97.3 mg/kg/day) starting two weeks after BDL-surgery (‘week 2’). In vivo 1H-MRS was performed on a 9.4 Tesla MRI system. Changes in metabolites were studied in the hippocampus (2 × 2.8 × 2 mm3) using SPECIAL2 sequence (TE = 2.8 ms). Metabolite concentrations were estimated by LCModel using water as internal reference. Open field test was performed at week 4, 6 and 8 to evaluate motor activity.4

RESULTS: Plasma measurements of bilirubin confirmed the presence of CLD in all groups of rats. They displayed similar ammonium concentration across groups (Figure 1a). IHH-MRS revealed some significant differences between the ‘high-dose rifaximin’ group at week 6 and at week 8, both in absolute value and relative to week 2 (+42% vs +118% at week 8, Figure 1b). Moreover, a decrease of glutamine was observed between week 4 and week 6 in the ‘high-dose rifaximin’ group (−10%), contrary to the non-treated group (Figure 1b). Also, in the ‘high-dose rifaximin’ group, decreases in the following metabolites were less pronounced during the time course of the study: myo-inositol, taurine, glutamate, ascorbate, creatine, total creatine (Figure 1c).

CONCLUSIONS: While rifaximin at human dose appeared to have an effect only at the early stages of the disease, a higher dose gave stronger positive effects on the neurometabolic profile. Importantly, no differences between the groups were observed in behavioural tests, but the ‘high-dose rifaximin’ rats had the tendency to move less. It is therefore possible that such a high dose of antibiotics also leads to some undesirable side-effects such as electrolyte abnormalities or inherent drug toxicity.5,6

REFERENCES
BACKGROUND: Chronic hepatic encephalopathy (HE) is a severe complication of chronic liver disease (CLD), and finding the right treatment to reduce HE episodes before liver transplant remains a challenge. Both rifaximin (non-absorbable antibiotic) and probiotics are currently used to reduce HE symptoms, but their precise effect on brain metabolites has never been studied. Our aims were: 1) to assess in vivo and longitudinally the effect of the combination of probiotics and rifaximin on bile duct ligated (BDL) rats in different brain regions; and 2) to compare these results with both non-treated (n = 17) and rifaximin-only treated rats (n = 12).1,2

METHODS: In vivo 1H-MRS at 9.4 Tesla combined with biochemical tests (plasma NH₄⁺, bilirubin) and microbiota analysis were performed on adult Wistar rats (n = 9) before BDL1,2 (week 0) and at weeks 2, 4, 6 and 8 after surgery. Evolution of metabolites was studied using the SPECIAL sequence (TE = 2.8 ms) in the hippocampus (2.8 × 2.8 × 2 mm³) and cerebellum (2.5 × 2.5 × 2.5 mm³). Metabolite concentrations were estimated using LCModel and water as internal reference. Probiotics administration (VIVOMIXX®, 60 billion bacteria/kg of rat) started two weeks before BDL-induced chronic liver disease (CLD) in children, Department of Pediatrics, University Hospitals Geneva, Switzerland; 3Service of Clinical Chemistry, University Hospital of Lausanne, Lausanne, Switzerland; 4Service of Clinical Chemistry, University Hospital of Lausanne, Lausanne, Switzerland; Center for Biomedical Imaging (CIBM); 5École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland; 6Swiss Center for Neuroinformatics, University of Geneva, Switzerland; 7Sapienza Universita di Roma, Rome, Italy.

RESULTS: All rats displayed the characteristic rise in plasma bilirubin, regardless of treatment group, as well as a similar ammonium increase (Figure 1a). The characteristic pattern of chronic HE was observed (Figure 1c): a gradual increase of brain glutamine followed by a gradual decrease in the other brain osmotolites (myo-inositol, taunine, total choline) and a later decrease of glutamate and creatine. The combination of probiotics and rifaximin improved some of the neuro-metabolic changes associated with CLD at early stages of HE (week 4) in the cerebellum: the probiotics + rifaximin group showed a lower increase of brain glutamine (+33% vs +66%, Figure 1c) and a smaller decrease of creatine (~3% vs ~14%). In the hippocampus, rats receiving both probiotics and rifaximin exhibited a smaller increase in brain glutamine even at week 8 after BDL compared to non-treated rats (+99% vs +136%, Figure 1d) and a smaller decrease in brain myo-inositol and glutamate (~20% vs ~30% and ~7% vs ~33%, respectively). Also, bifidobacteria concentration was slightly higher in the probiotics + rifaximin group at week 8 (Figure 1b). Finally, the administration of rifaximin associated with this probiotic showed more beneficial effects than rifaximin only, and both could be used to maintain a balanced microbiota and may provide opportunities for reducing the spread of antibiotic resistances.

CONCLUSIONS: To conclude, some promising changes were induced in the neurometabolic profile of BDL-rats who were treated with this specific probiotic and rifaximin (glutamine, myo-inositol, creatine and glutamate).

Clinical outcomes from univariate and multivariate analyses comparing rifaximin-treated and rifaximin-naive patients on the liver transplant waiting list.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(95% CI)</td>
<td>(95% CI)</td>
</tr>
<tr>
<td>All-cause admissions/year</td>
<td>-3.55 (-6.55 to -0.55)</td>
<td>-3.10 (-6.00 to -0.20)</td>
</tr>
<tr>
<td></td>
<td>p = 0.021</td>
<td>p = 0.037</td>
</tr>
<tr>
<td>Days to readmission</td>
<td>+82 (48–117)</td>
<td>+71 (3–193)</td>
</tr>
<tr>
<td></td>
<td>p = 0.025</td>
<td>p = 0.040</td>
</tr>
<tr>
<td>Admissions with sepsis/year</td>
<td>-0.97 (-2.27 to -0.33)</td>
<td>-0.49 (-1.75 to -0.56)</td>
</tr>
<tr>
<td></td>
<td>p = ns</td>
<td>p = ns</td>
</tr>
<tr>
<td>Admissions with complications of ascites</td>
<td>-1.70 (-3.00 to -0.4)</td>
<td>-1.77 (-3.07 to -0.47)</td>
</tr>
<tr>
<td>including SBP/year</td>
<td>p = 0.010</td>
<td>p = 0.008</td>
</tr>
<tr>
<td>Admissions with acute variceal bleeding/yr</td>
<td>-0.89 (-1.59 to -0.19)</td>
<td>-0.81 (-1.52 to -0.10)</td>
</tr>
<tr>
<td></td>
<td>p = 0.014</td>
<td>p = 0.026</td>
</tr>
<tr>
<td>Admissions with overt hepatic encephalopathy/yr</td>
<td>-0.01 (-0.81 to 0.79)</td>
<td>-0.07 (-0.95 to 0.81)</td>
</tr>
<tr>
<td></td>
<td>p = ns</td>
<td>p = ns</td>
</tr>
<tr>
<td>Length of hospital stay (days)</td>
<td>-5.74 (-12.25 to 1.06)</td>
<td>-6.35 (-12.85 to 0.15)</td>
</tr>
<tr>
<td></td>
<td>p = ns</td>
<td>p = ns</td>
</tr>
<tr>
<td>Intensive care admissions/year</td>
<td>-0.46 (-1.66 to 0.74)</td>
<td>-0.04 (-1.18 to -1.10)</td>
</tr>
<tr>
<td></td>
<td>p = ns</td>
<td>p = ns</td>
</tr>
<tr>
<td>Length of intensive care stay (days)</td>
<td>-1.40 (-3.80 to 1.20)</td>
<td>-1.15 (-3.46 to 1.18)</td>
</tr>
<tr>
<td></td>
<td>p = ns</td>
<td>p = ns</td>
</tr>
<tr>
<td>Requirement for prioritisation on the waiting list (odds ratio)</td>
<td>0.34 (0.0 to 0.72)</td>
<td>0.29 (0.0 to 0.71)</td>
</tr>
<tr>
<td></td>
<td>p = 0.030</td>
<td>p = 0.037</td>
</tr>
<tr>
<td>Mortality on the waiting list (odds ratio)</td>
<td>0.66 (0.0 to 1.61)</td>
<td>0.40 (0.0 to 1.09)</td>
</tr>
<tr>
<td></td>
<td>p = ns</td>
<td>p = ns</td>
</tr>
</tbody>
</table>

(adjusted effect estimate 71, 95% CI 3–140 days, P = 0.037) and reduced likelihood of requirement for prioritisation on the waiting list (odds ratio 0.29, 95% CI 0.89–0.93, P = 0.037).

CONCLUSION: Rifaximin prescribed for HE in patients listed for liver transplantation improved outcomes on the waiting list with a significant reduction in admissions related to spontaneous bacterial peritonitis, ascites and AVB and indicating potential beneficial impacts of rifaximin beyond HE in ACLD.

**P: 39 Junior Investigator**

Modulation of the Urea Cycle Function by Toll-like Receptor 4 Signaling: A Potential Novel Therapeutic Target for Hyperammonemia and Hepatic Encephalopathy

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**BACKGROUND:** Lipopolysaccharide (LPS) and ammonia act synergistically in mediating the severity of hepatic encephalopathy (HE) in cirrhosis. Although LPS results in neuroinflammation, it is not clear whether it induces hyperammonemia (HA) contributing to HE. This study addressed the following questions: 1) Does LPS worsen HA in cirrhosis? 2) Does treatment with an antagonist (TAK242) of the LPS receptor, toll-like receptor 4 (TLR4), prevent HA? 3) Is a TLR4 knock-out (TLR4-/-) animal protected from HA? If so, what is the underlying mechanism? Do they have a more effective urea cycle?

**METHODS:** Study 1: Sprague Dawley rats were treated with LPS (0.025 mg/kg, ip.) 4 weeks after biliary duct ligation (BDL). 4 groups of rats were studied: sham (n = 4), BDL (n = 4), BDL+LPS (n = 6) and BDL+TAK242 (10 mg/kg, ip.) 3 hours before LPS injection (n = 7). Study 2: 4 groups of mice were studied: wild type control (WTC, n = 7), WT with HA (WTH, n = 10), TLR4-/- control (TLR4-/-C, n = 10) and TLR4-/- with HA (TLR4-/-H, n = 10). HA was induced by adding 0.28M ammonium chloride to drinking water for 3 days. For both studies, plasma ammonia and liver gene expression (qPCR, data shown as 2^-ΔΔCT compared to sham/WT) of the 5 urea cycle enzymes (UCEs) were assessed. For study 2, protein expression of the key, rate-limiting enzyme carbamoyl phosphate synthetase 1 (CPS1) was also assessed (Western Blot, immunohistochemistry).

**RESULTS:** Study 1: There was a stepwise increase in plasma ammonia throughout sham, BDL and BDL+LPS groups (P < 0.001). Pre-treatment with TAK242 prior to LPS injection in BDL rats was
associated with a reduction in plasma ammonia (P < 0.01, Figure 1a) and a higher coma-free survival rate (100% vs. 15%). Gene expression of all UCEs showed a stepwise decrease throughout sham, BDL and BDL+LPS (all P < 0.05), which was prevented by TAK242 (all P < 0.05). This was most pronounced for CPS1, for which expression levels in the TAK242-treated group were restored to that of the sham animals (Figure 1b). Study 2: In TLR4-/- mice, the increase in plasma ammonia was less compared to WT mice (P < 0.001). Although no significant changes were found for gene expression of UCEs between groups, protein expression of CPS1 was significantly higher in TLR4-/- mice as compared to WT mice, as shown by both Western Blot and immunohistochemistry.

**CONCLUSIONS:** These data suggest that TLR4 signaling contributes to the development of hypokalemia.

**METHODS:** Female Wistar rats were fed a K−free diet for 13 days. Half of the rats were then repleted with K+ for one week following depletion. K+ -depleted and -repleted rats were compared to free-fed and pair-fed controls. We examined the urea cycle enzyme mRNAs and proteins in liver tissue, the in vivo Capacity of Urea-Nitrogen Synthesis (CUNS) and plasma ammonia concentrations.

**RESULTS:** The diet induced hypokalemia of 1.9 ± 0.4 mmol/L compared to pair-fed controls (3.6 ± 0.2 mmol/L). Muscle and kidney tissue potassium concentrations were decreased, but unchanged in liver tissue. Gene expression of albumin and two out of four free urea cycle enzymes were moderately decreased, whereas protein expressions of albumin, the urea cycle enzymes, and glutamine synthetase were normal. However, CUNS was reduced by 33%. Plasma ammonia concentrations were eight-fold elevated to 235 (95% CI. 194–287) µmol/L compared to pair-fed controls 29 (95% CI. 26–32) µmol/L. Repletion of potassium normalized the changes.

**CONCLUSIONS:** Hypokalemia markedly increased plasma ammonia concentrations. The capacity for urea synthesis was impaired, but only moderately so, and further studies are needed to fully explain the causes of hyperammonemia.

**REFERENCES**
1. Faust F, Schubert S. Protein synthesis is the most sensitive process when potassium is substituted by sodium in the nutrition of sugar beet (Beta vulgaris). Plant physiology and biochemistry: PPR 2016;107:237–247. doi:10.1016/j.plaphy.2016.06.009.

**P: 41 Junior Investigator**

**Precipitants of Hepatic Encephalopathy, In-hospital Mortality and Its Predictors**
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**BACKGROUND:** Hepatic encephalopathy (HE) is a common complication of liver dysfunction, including acute liver failure and liver cirrhosis. HE presents as a spectrum of neuropsychiatric symptoms ranging from subtle fluctuating cognitive impairment to coma. It is a significant contributor of morbidity in patients with liver disease. Common culprits include gastrointestinal bleeding, infection, constipation, hypokalemia, hyponatremia, and medications such as opiates and benzodiazepines. This study aimed to evaluate the change in common precipitants of HE, in-hospital mortality and its predictors.

**METHODS:** This descriptive study was conducted in Hepatology division of Asian Institute of Medical Sciences, Hyderabad, Pakistan from October 2018 to April 2019. A total of 82 cirrhotic patients with HE were included in the study. Frequency of precipitating factors were determined and in-hospital mortality was evaluated.

**RESULTS:** A total of 82 cirrhotic patients with mean age 52.31 years, with males 62.2%. Common etiology for cirrhosis was HCV 64.63%, 56.09% were CTP B, 36.58% patients had MELD in between 11-15. According to West Haven criteria most common grades were Grade II 52.43%, Grade III 17% and Grade IV 16%. Most common precipitants were hyponatremia 18%, constipation 12%, hypokalemia 5%, infections 10%, variceal upper GI bleeding 4%, 24% had mixed precipitants and in 27%
patients no precipitant found. 42.7% had Acute Kidney Injury (AKI) mostly associated with electrolyte imbalance. 70.7% improved and discharged, 11% were expired, 18.3% discharged on request. AKI, SBP and Shock were associated with high mortality.

CONCLUSIONS: Electrolyte imbalance, constipation and infections were leading causes of HE. AKI was independent predictor of HE and in-hospital mortality along with SBP and shock.

P: 42  Junior Investigator

To Assess Frequency of Hepatic Encephalopathy in Spontaneous Bacterial Peritonitis Patients

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BACKGROUND: Hepatic encephalopathy (HE) is a commonly encountered complication in cirrhosis. The incidence of HE ranges from 2% to 20% per year in patients with liver cirrhosis. HE is associated with increased morbidity and mortality as well as significant utilization of health care resources. Most cases of sign and severity of HE are caused by gastrointestinal bleeding, infection, concomitant, electrolyte imbalance and medications. Spontaneous bacterial peritonitis (SBP) is an independent predictor of HE. We evaluated frequency of HE in SBP patients.

METHODS: This cross-sectional study was conducted at the Gastro-hepatology section of Asian Institute of Medical Sciences in Hyderabad, Pakistan from April 2017 to March 2019. 120 Patients with paracentesis-proven SBP [Absolute Neutrophils counts (ANC) ≥ 2500/mm3] and clinically assessed HE were examined. Most common serology was HCV (61.7%). 21 were CTP B (17.5%) and III (29.33%) and IV (21.33%). Mean ANC 5086, 96 (80%) were PPI users and 64.58% have HE.

RESULTS: A total of 120 patients of SBP with mean age 47.80 years, with 88 (73.3%) males and 32 (26.7%) females were examined. Most common serumology were HCV (61.7%). 21 were CTP B (17.5%) and III (29.33%) and IV (21.33%). Mean ANC 5086, 96 (80%) were PPI users and 64.58% have HE.

CONCLUSIONS: Hepatic encephalopathy has strong association with SBP and PPI use. Screening for asymptomatic SBP in all patients with HE should be considered and over-the-counter PPI use should be restricted.

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A Sensitive and Convenient Protocol for Determining Brain Water Content in Rats using a Moisture Analyzer

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BACKGROUND: Brain water content represents a major endpoint in studies of hepatic encephalopathy and liver failure. However, none of the current methods for evaluating brain water content is simple and inexpensive. The Moisture Analyzer (Mettler Toledo) has been used in the clinical setting for measuring body fluids and tissue moisture content. We evaluated the feasibility of using this instrument for measuring brain water content in rats.

METHODS: Female Sprague-Dawley rats (248 ± 41 g bw) undergoing a 3-hour protocol for evaluating brain water content using the moisture analyzer was a convenient and sensitive method for measuring brain water content in rats.

RESULTS: Water content was measured in 40 rats. The H-score (200 ± 15) and creatinine (5.0 ± 0.5 μM/L) were significantly higher in the ACLF group than in the control group (H-score 130 ± 15, P < 0.05; creatinine 3.5 ± 0.5 μM/L, P < 0.05). Measuring time of 15 minutes per sample.

CONCLUSIONS: This study demonstrates non-canonical inflammasome activation and as a feature of ACLF concomitant to HE, resulting in activation of the target protein GSDMD in the liver. The non-canonical inflammasome activation is enhanced in cirrhosis, and deletion of Casp11 is shown to be protective against the onset of HE in ACLF.

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Non-canonical Inflammasome as a Driver of Hepatic Encephalopathy in a Mouse Model of Acute-on-Chronic Liver Failure

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BACKGROUND: Hepatic encephalopathy (HE) is a key feature of Acute-on-chronic liver failure, a common cause of mortality in cirrhosis. ACLF is characterized by multi-organ failure and disease progression is associated with elevated levels of circulating bacterial endotoxin (LPS), and increased non-antipotic hepatocyte cell death. The non-canonical inflammasome is a LPS-sensing pathway, mediated by caspase-11 which, when activated, leads to cleavage of the cytotoxic protein Gasdermin D (GSDMD) and subsequent pyroptosis - a form of non-apoptotic cell death. The aim of this study was to determine the role of this non-canonical inflammasome in the onset of HE in cirrhosis and ACLF.

METHODS: CIRHOS was induced in male C57BL/6 mice by CEXA garage, twice weekly for 10 weeks. Subsequently, mice underwent ip injection of LPS to induce AC. Brain tissue water content, plasma ALT and creatinine were measured by standard techniques. GSDMD cleavage was assessed by Western blot for full length and N-terminal protein. Caspase-11 activation was measured by colorimetric assay in liver protein extract. Mice deficient in Casp11 showed protection against HE upon injection of AC. The form of reduced brain swelling, and levels of circulating ALT and creatinine.

RESULTS: All C4H + LPS mice showed features of ACLF following LPS injection, with increases in brain weigh (79.9 ± 0.2 vs 78.9 ± 0.1%, P < 0.01), plasma ALT (102.0 ± 8.9 vs 79.2 ± 6.2 μM/L, P < 0.05) and creatinine (48.5 ± 5.6 vs 34.8 ± 1.3 μM/L, P < 0.05) at hours compared to control (Figure 1a). Cleavage of hepatic GSDMD (ratio N-terminal: full length GSDMD 7.7 ± 1.1 vs 0.2%, P < 0.001) and activation of caspase-11 (2 fold induction, P < 0.05) (Figure 1b) was seen in the ACLF group at 4 hours compared to control. Caspase11-/- mice were protected against AC, with reduced brain weigh (78.3 ± 0.2% vs 78.3 ± 0.2%, P < 0.05), plasma ALT (530 ± 144 vs 172 ± 18 μM/L, P < 0.05) and creatinine (179 ± 1.4 vs 15.7 ± 1.0 μM/L, P < 0.05) at hours after LPS injection compared to wild-type controls (Figure 1c).

CONCLUSIONS: This study demonstrates non-canonical inflammasome activation and as a feature of ACLF concomitant to HE, resulting in activation of the target protein GSDMD in the liver. The non-canonical inflammasome activation is enhanced in cirrhosis, and deletion of Casp11 is shown to be protective against the onset of HE in ACLF.

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Increased Iba-1 Expression in an Autopsied Brain Samples of ALF & Chronic Liver Disease Patients With Hepatic Encephalopathy

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BACKGROUND: Systemic inflammation both in acute liver failure (ALF) and patients with cirrhosis and hepatic encephalopathy (HE) leads to microglial activation and an increase in oxidative stress. Iba1 has been found to be a highly specific marker expressed in microglial cells in animal models. We studied Iba-1 expression in the brains of patients with ALF and CLF with hepatic encephalopathy and the control patients without any neurological disease.

METHODS: Autopsied frontal cortex samples were obtained from 5 patients with ALF due to viral hepatitis (HEV, HBV) and/or tumor liver injury (ALT induced) or mushrooms poisoning. 7 patients with cirrhosis and HE and 7 controls were obtained. Expression of Iba-1 mRNA was investigated by real-time PCR using appropriate molecular probes and protein expression was assessed using immunohistochemistry using commercially available polyclonal antibodies and measure the intensity with the help of H-score.

RESULTS: Iba-1 immunostaining highlights the microglial cells in the grey matter of the brain in ALF patients (H-score = 2000) as compared to the patients with cirrhosis and HE (H-score = 450) and to controls (H-score = 300). ALF grey matter specifically highlights the microglial nodules, and faint cyttoplasmic hue is also noted in the neurons. Gene expression studies show the increased fold change in the Iba-1 in ALF (fold change = 4.08, P = 0.011) and in patients with cirrhosis and HE (fold change = 11, P = 0.84) patients when compared with controls, though not statistically significant.

CONCLUSIONS: These results demonstrated the increased microglial activation as well as the number of microglial cells in the ALF patients that can contribute to neuroinflammation.

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Ammonia Is an Independent Biomarker of Poor Outcomes in Patients With Advanced Cirrhosis on the Transplant Waiting List

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BACKGROUND: Ammonia plays a pivotal role in the development of hepatic encephalopathy (HE) and brain oedema in acute liver failure and is a poor prognostic biomarker. However, its utility as
Figure 1

A

- **Brain swelling (%) water**
  - $p=0.053$
  - CCl$_4$ vs. CCl$_4$ + LP4

- **ALT (U/L)**
  - $p=0.056$
  - CCl$_4$ vs. CCl$_4$ + LP4

- **Creatinine (umol/L)**
  - $p=0.0504$
  - CCl$_4$ vs. CCl$_4$ + LP4

B

- **Caspase 11 activity**
  - $p<0.05$

  - Normal vs. CCl$_4$

C

- **Brain swelling (%) water**
  - $p<0.05$
  - WT vs. Casp11$^{-/-}$

- **ALT (U/L)**
  - WT vs. Casp11$^{-/-}$

- **Creatinine (umol/L)**
  - WT vs. Casp11$^{-/-}$

[44]
In this retrospective study, arterial ammonia at the time of listing for liver transplanted patients, ammonia levels correlated with the presence of post-op complications (ammonia measured at the time of assessment for transplant. All patients were closely followed up until death.

METHODS: A retrospective observational study of 300 sequential cirrhotic adult patients listed for liver transplantation between January 2015 and December 2018 was undertaken. All patients had an arterial ammonia measured at the time of assessment for transplant. All patients were closely followed up until death or transplantation. The main outcomes were hospital admissions (and number of organ failures), infection and mortality. Chronic Liver Failure-Sequential Organ Failure Assessment criteria were used to determine the presence of organ failures. For the transplanted patients, post-op complications were recorded.

RESULTS: 200 (66.7%) were male with mean age of 54.29 ± 10.4. Mean follow-up time was 722.6 days. 266 (88.7%) patients were transplanted, 15 (5.0%) were de-listed for being too sick for transplant or following clinical improvement, 14 (4.7%) died on the list and 5 (1.6%) were still active on list. 97 (32.3%) patients were hospitalized on the waiting list and 60 (20%) had evidence of at least one infection. On multivariate analysis, ammonia correlated with hospitalisation (P < 0.001), infection (P < 0.001) and all-cause mortality (P = 0.0013). Of the patients that had at least one hospital admission, ammonia was an independent predictor of the presence of acute-on-chronic liver failure (ACLF) (P = 0.01811). For the transplanted patients, ammonia levels correlated with the presence of post-op complications (P < 0.001).

CONCLUSIONS: In this retrospective study, arterial ammonia at the time of listing for liver transplantation was an independent predictor of hospitalisation, ACLF, infection and mortality. These data suggest that blood ammonia may be an important determinant of wait-list survival and further prospective studies are warranted.

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Junior Investigator

Risk of Recurrent Hepatic Encephalopathy (HE) in Patients With Liver Cirrhosis: A German Registry Study

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BACKGROUND: Patients with Hepatic Encephalopathy (HE) show a low quality of life, recurrent hospitalisations and an increased mortality. We aimed to assess the natural course of patients after a recent HE-episode under the conditions of the German health system, as respective data were not available.

METHODS: Fifteen sites from Germany - 8 of them liver transplant (LT) centers - took part in an observational prospective study including cirrhotic patients who had been hospitalised due to an acute episode of HE within 3 months before recruitment. Age < 18 years, no liver cirrhosis, malignancies and current hospitalization were exclusion criteria. Demographic and clinical data, health related quality of life (HRQoL) score SF-36, psychometric hepatic encephalopathy score (PHES) and critical flicker frequency (CFF) were assessed and monitored quarterly for one year. Primary endpoint was a novel clinical manifestation of HE necessitating hospital admission. Secondary endpoints were the combined endpoint of hospital admission for a novel HE episode and/or death, the dynamics of the West Haven Criteria (WHC) as well as changes in CFF, PHES and SF-36.

RESULTS: A total of 115 patients were recruited. Forty-four patients (12.4 %) died during the study period due to complications of liver cirrhosis other than HE. For 67 subjects follow-up data were available in accordance with the protocol. After a median of 113 days half of the per protocol cohort (N = 34) was re-admitted due to a recurrent manifestation of HE. The patient groups with and without re-hospitalization differed significantly regarding recruitment sites (LT centers vs no LT centers) (P = 0.005), interval from discharge to recruitment (P = 0.007), history of more than 4 HE relapses prior to recruitment (P = 0.029), SF-36 mental score (P = 0.046) and PHES ≤ −3 (P = 0.004), whereas CFF, clinical (e.g. MELD score, WHC grade) and laboratory data did not differ. Of note, CFF performance was correct only in about half of both, the total and the per protocol group. Patients with incorrect CFF performance had worse PHES results than those with valid CFF measurement. Multiple logistic regression analysis revealed a PHES test result of ≤ −3 as an independent risk factor for re-hospitalization (P = 0.046).

CONCLUSIONS: Mortality in our cohort is comparatively low. Despite advanced treatment strategies, patients with a history of HE are still sincerely jeopardized to develop recurrent clinical HE. The PHES test appears useful for detection, monitoring and stratification of recurrent HE. Patients with PHES ≤ −3 at baseline had an increased risk of deterioration.

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Junior Investigator

Nonselective Beta-blocker Use Is Associated With Increased Hepatic Encephalopathy-Related Readmissions in Patients With Cirrhosis

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BACKGROUND: Hepatic Encephalopathy (HE) is a reversible syndrome of impaired brain function that is associated with frequent hospitalizations and decreased survival in cirrhotic patients. Nonselective beta-blockers (BBs) have been recommended to reduce the risk. Yet, it is unknown if BB use is associated with an increased risk of HE-related hospitalizations. Therefore, we assessed the risk of HE-related hospitalizations.

METHODS: Using a retrospective cohort study design, we identified patients with cirrhosis who received a diagnosis of HE within 1 year of starting a BB. We included patients initiating a BB within 3 months prior to the index date (2013-2017) and followed them up to 2017. We compared hospitalization rates for HE-related hospitalizations among patients who did and did not receive BBs. We defined HE-related hospitalizations as hospitalizations for a HE episode with or without a subsequent readmission for any cause. We calculated the Kaplan-Maier survival curves to visualize the time to first HE-related hospitalization among patients with and without BBs. Competing risks analysis was performed to determine the rate of HE-related hospitalizations.

RESULTS: 2,197 patients with cirrhosis were included in the study. Of these, 623 received a BB (32.8%) and 1,574 did not receive a BB (67.2%). The median follow-up time was 426 days. The rate of HE-related hospitalizations was 55% for patients receiving BBs and 51% for patients not receiving BBs. The median time to first HE-related hospitalization was 63 days for patients receiving BBs and 78 days for patients not receiving BBs. The overall rate of HE-related hospitalizations was 0.24 events per patient-year (95% CI 0.20 to 0.29) and 0.22 events per patient-year (95% CI 0.20 to 0.26) respectively. The risk of HE-related hospitalizations was higher for patients receiving BBs (Hazard Ratio 1.02, 95% CI 0.97 to 1.06, P = 0.26).

CONCLUSIONS: Nonselective BBs are associated with an increased risk of HE-related hospitalizations in patients with cirrhosis.
beta-blockers (NSBB) are the mainstay of pharmacologic treatment for portal hypertension and prevention of variceal bleeding. Due to their effects on hepatic blood flow, we hypothesized that NSBB use would decrease portal flow, leading to increased HE-related hospitalizations independent of liver disease severity. This study was done to assess the effect of NSBB use on HE-related readmissions.

METHODS: We examined all the patients with cirrhosis admitted at Baylor University Medical Center between January 2013 and July 2018. The outcome measure of HE-related readmissions was HE-related re-hospitalizations; Hazard ratio was 2.82 (95% confidence interval: 1.81–4.41). Patients and controls had similar driving duration of driving 44 (42, 50) vs 45 (24, 51), P = 0.48. On MRI driving simulation: Mean activations: As the driving task complexity increased from No-traffic to Traffic to Traffic+Distractor states, we observed a shift of increased activation from parietal (precuneus, supramarginal and angular gyrus) and visual (lingual gyrus, V1 and V2) to frontal (dorsolateral prefrontal cortex, orbitofrontal cortex), error detection and conflict monitoring (anterior cingulate cortex), attentional resource allocation (paracingulate gyrus), visual attention regulation (superior parietal lobule), inhibitory control (left middle frontal gyrus) and regions associated with regulation of voluntary movement (left pallidum, putamen) (Figure 1c).

CONCLUSIONS: Using MRI-compatible driving simulation, patients with cirrhosis demonstrated suppressed attention regulation circuits and sensorimotor control compared to controls, which worsened when distractors such as cellphone use were included. This is likely the neural basis for impaired driving skills in cirrhosis.
BACKGROUND: Hepatic encephalopathy (HE) is a neuropsychiatric syndrome, a major complication of chronic liver disease (CLD/cirrhosis). The primary cause of hospital admissions for cirrhotic patients is an overt episode of HE. Precipitating factors of HE frequently lead to an increase in blood ammonia. Patients who have experienced multiple episodes of HE are associated with persisting neurological complications post-liver transplantation. Currently, the impact of HE episodes on neurological integrity is unknown. We hypothesize that multiple episodes of HE will accelerate and/or intensify neurological deterioration. To date, an animal model of episodic HE is lacking. Therefore, our goal was to characterize an animal model of episodic HE (pre-coma; loss of righting reflex) every 4 days starting 3 weeks post-BDL surgery as vehicle for non-episodic groups. Two days following the last HE episode, we assessed motor-coordination (Rotabold), anxiety (elevated plus maze, EPMt), as well as short-term and long-term memory (novel object recognition) in all groups. Upon sacrifice, plasma ammonia was measured.

RESULTS: The concentration of ammonia required to induce an episode of overt HE in BDLC rats lessened with each subsequent episode, ranging from 7 to 4.5 mmol/kg. Short-term memory (P < 0.05) and motor-coordination (P < 0.05) were impaired in both non-episodic and episodic BDLC groups compared to respective Sham-operated controls. Long-term memory impairment (P = 0.06) and increased anxiety (+40%, P < 0.05) were exclusively found in episodic BDLC rats compared to non-episodic BDLC rats. Moreover, there was an increase in blood ammonia (>30.4%, P = 0.06) in episodic compared to non-episodic BDLC rats, suggesting that although episodic-BDLC rats recover from each HE episode, baseline (pre-episode) ammonia remain higher than non-episodic BDLC rats.

CONCLUSIONS: Cumulative HE episodes escalate neurological impairments in cirrhotic-BDLC rats. Thus, this new episodic HE model represents a good approach to explore the pathophysiological mechanism arising from multiple episodes, as well as further investigate whether higher hyperammonemia and/or increased brain sensitivity to ammonia is responsible for more complex neurological manifestations in episodic HE, rats. Moreover, this model is an excellent platform to investigate novel therapies to prevent/treat episodic HE.

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Junior Investigator

Extent of Sarcopenia Does Not Correlate With Degree of Minimal Hepatic Encephalopathy in Patients on Treatment for Hepatic Encephalopathy
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BACKGROUND: Minimal hepatic encephalopathy affects over half of the patients with cirrhosis. It leads to deterioration of quality of life due to cognitive deficit. Both presence of sarcopenia and postprandial somatic symptoms have been implicated in development of minimal hepatic encephalopathy. We retrospectively assessed the significance of sarcopenia and presence of postprandial symptoms in patients with minimal hepatic encephalopathy.

METHODS: Patients with cirrhosis of liver attending the liver clinic at University of Padua underwent a detailed elective outpatient assessment for neurocognitive function. This included Psychometric Hepatic Encephalopathy Score (PHES), computer based tests, and EEG. We then selected patients who underwent a computed tomography for any reason within 3 months of the neurocognitive assessment. The degree of sarcopenia and presence of postprandial symptoms was evaluated on the scan. Presence of more than one shunt was accounted and adjusted for appropriately using a physiological approach previously described.

RESULTS: A total of 120 patients had a CT scan within 3 months of the neurocognitive assessment. Of these, 100 were found to be suitable for analysis. Mean age of the patients was 59 ± 9.6 yrs and 73% were males. Median Child Pugh score was 8 (IQR 6–10) and MELD was 12 (IQR 10–18). Alcohol and viral etiologies responsible for 40% and 39% cases respectively. Based on Psychometric Hepatic Encephalopathy Score (PHES), 39% had minimal hepatic encephalopathy. Medication details were available for 87 patients, 70% of whom were on some form of treatment of hepatic encephalopathy with lactulose, antibiotics, or probiotics. The degree of cognitive dysfunction (PHES) correlated significantly with education (r = 0.33), shunt size (r = -0.45), and degree of hyperammonemia (r = -0.48), but not with sarcopenia or degree of hepatic dysfunction. Correlation between total effective shunt size and PHES was more pronounced in women (r = -0.56). Upon multivariate analysis, none of these variables predicted PHES. EEG mean dominant frequency correlated significantly with total effective shunt size (r = -0.30), ammonia (r = -0.34), and Child-Pugh score (r = -0.38). None of the parameters significantly correlated with critical flicker frequency. There was no difference in the degree of sarcopenia among patients with and without MHE or history of overt HE. Moreover, degree of sarcopenia did not correlate with PHES, critical flicker frequency, ammonia level, or Child-Pugh score. Effective size of postprandial shunt was higher in patients with MHE.

CONCLUSIONS: Among patients with cirrhosis on anti-encephalopathy treatment, presence of sarcopenia does not correlate with neurocognitive parameters. Presence of large postprandial shunts correlated with neurocognitive impairment.

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Junior Investigator

Diagnosis of Covert Hepatic Encephalopathy Is Influenced by Multiple Non-cognitive Variables That Varies by Testing Strategy
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Covert hepatic encephalopathy (CHE) is a serious complication of cirrhosis that manifests as an amnestic cognitive dysfunction. Diagnosis is based on examining cognitive functions, but results are influenced by multiple non-cognitive factors such as sleep and medications but data regarding the influence of other non-cognitive covariates is limited. We aimed to examine the potential non-cognitive variables that could influence testing on the psychometric hepatic encephalopathy score (PHES) and the encephalappp stroop.

METHODS: Patients between ages 20-75 years were enrolled from clinic between 2012–2018. Those with severe uncontrolled psychiatric comorbidities were excluded as were those actively abusing alcohol or drugs. All patients underwent testing for CHE based on 2 testing strategy as recommended by the AASLD/EASL guidelines. Demographics and details of comorbidities were collected. CHE was diagnosed based on published norms. Appropriate t tests and logistic regression were done. Dependent variable was CHE on PHES and Stroop.

RESULTS: We enrolled a total of 257 patients with mean age 61.1 ± 8.3, 72% were males, HCV was the predominant etiology 96 (37.3%). Median BMI was 29 (26, 34). Median education was 13 (12, 16) years. Median MELD was 11 (7.25, 15) with median childs score of 6 (5, 8). Charlson comorbidity score (CCI) was 5 (4, 6). Eighty-five (33%) had a history of prior OHE and were on lactulose (13), rifaximin (9) or both (63), 95 (37%) had ascites with 58 (61%) controlled on diuretics and 17 (18%) had a history of SBP. 41 (16%) had a history of variceal bleeding. In terms of comorbidities 34.2% had Diabetes Mellitus, 58% had Hypertension, 11% had Coronary artery disease, 14% had hypothyroidism, 2% had CHF, 2.3% had COPD, 30% had depression and 87% were controlled on medications. 2.3% had post traumatic stress disorder (PTSD) and were on medications. 14% were on chronic narcotic medications. On cognitive testing 109 (42.4%) had CHE with a median PHES score of 3 (7, 0) whereas 206 (80%) tested positive on encephalapp stroop. On univariable analysis hypertension was found to be significant only for stroop. On multivariable analysis age and prior OHE were predictive for both tests but for the Encephalapp stroop hypertension was found to be independently predictive (Table 1).

CONCLUSION: The diagnosis of CHE can be influenced by other non-cognitive variables and these vary between individual testing strategies probably due to the differential effects that these systemic conditions have on cerebral/subcortical functions. Physicians must take into consideration these covariates while interpreting CHE testing based on these 2 tests.

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Decreased Cognitive Performance Is Associated With Reduced Resting State Connectivity and Gray Matter Atrophy in Patients With Minimal Hepatic Encephalopathy

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CONCLUSIONS: Decreased cognitive performance is associated by reduced rs-FC and GM atrophy in MHE patients. These changes could have predictive value for detecting MHE. Alterations in the FC of the hippocampal system could contribute to learning and long-term memory impairments in MHE patients. This study shows the association between alterations in learning and long-term memory and structural and FC disturbances in hippocampal structures in cirrhotic patients. Supported by Ministerio Economia, Industria y Competitividad - Instituto Salad Casals III (FIS PI15/00035; FIS PI18/00150) to CM, Conselleria Educación Generalitat Valenciana (PROMETEO/2014/033, PROMETEO/2018/051 to VF), CM, ACFI/2018/284 to [JC], co-funded with European Regional Development Funds (ERDF).

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Evaluation of Cognitive Dysfunction in Animal Models and Relatability to Human Disease
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ABSTRACT: Animal models are essential to investigate the mechanisms responsible for the cognitive and motor alterations in minimal or clinical hepatic encephalopathy (HE). The characterization of these mechanisms allows identifying new therapeutic targets which modulation may improve neurological function in patients. Animal models also allow testing if treatments directed to modulate these targets improve cognitive and motor function. These studies require evaluation of cognitive and motor function in animal models. It is important to understand which tests can be performed in rodents, which cognitive and motor aspects are evaluated by the tests and how they can be related to cognitive and motor dysfunction in the patients. The earliest alterations in patients with minimal HE (MHE) include attention deficits, psychomotor slowing, impairment of visuo-motor and bimanual coordination, of working memory, spatial memory, long-term memory, spatial orientation, verbal learning, concentration, balance and equilibrium, associated with increased falls. To understand the mechanisms involved in each type of cognitive and motor alteration in MHE the behavioral tests in animal models should be designed to evaluate these neurological alterations by procedures that maximize the translatability to the human disease.

BACKGROUND: Patients with minimal hepatic encephalopathy (MHE) show mild cognitive impairment associated with cognitive alterations and changes in connectivity. We assessed the relationship of abnormalities of resting-state functional connectivity (rs-FC) and gray matter (GM) volume with cognitive alterations and biochemical parameters associated to MHE. We also evaluated the relationship between memory in MHE and structural and functional connectivity (FC) changes in the hippocampal system.

METHODS: Twenty-six cirrhotic patients without MHE (NMHE), 13 with MHE, and 24 controls were cognitively assessed with a battery of psychometric tests. Atrophy was determined using Voxel-Based Morphometry and rs-FC was assessed by ICA analysis. Receiver operating characteristic (ROC) curves was performed to assess the diagnostic utility of rs-FC and GM atrophy in the discrimination of patients with and without MHE. We also assessed the relationship between alterations in memory and the structural integrity and FC of the hippocampal system.

RESULTS: MHE patients showed significant decrease of GM volume and lesser degree of rs-FC in different networks related to attention and executive functions as compared to controls and NMHE. There is a progressive reduction in rs-FC in the default network with the progression of cognitive impairment. MHE patients showed GM reduction in right frontal lobe, right insula and right cerebellum compared to NMHE patients. Alterations in GM volume and rs-FC correlated with cognitive tests. MHE patients showed impairments in learning, memory, and recognition, compared to NMHE and controls. Cirrhotic patients showed reduced fimbria volume compared to controls. Larger volumes in hippocampus subfields were related to better memory performance in NMHE patients and controls. MHE patients presented lower FC between the L-presubiculum and L-precuneus than NMHE patients, and a reduced FC between L-presubiculum and subiculum seeds and bilateral precuneus, which correlated with cognitive impairment and memory performance.

DISCUSSION: MHE patients showed a milder cognitive impairment than NMHE. These changes could have predictive value for detecting MHE. Alterations in the FC of the hippocampal system could contribute to learning and long-term memory impairments in MHE patients. This study shows the association between alterations in learning and long-term memory and structural and FC disturbances in hippocampal structures in cirrhotic patients. Supported by Ministerio Economia, Industria y Competitividad - Instituto Salad Casals III (FIS PI15/00035; FIS PI18/00150) to CM, Conselleria Educación Generalitat Valenciana (PROMETEO/2014/033, PROMETEO/2018/051 to VF), CM, ACFI/2018/284 to [JC], co-funded with European Regional Development Funds (ERDF).
human situation and the utility to evaluate treatments to improve cognitive and motor impairment. Many functions are evaluated in humans using pencil or computerized tests such as number connection tests, repetition of series of words or numbers, naming colors, and other actions which can’t be reproduced exactly in animal models. Functions such as verbal learning can’t be evaluated in animal models. However, a good number of cognitive and motor processes impaired in patients with MHE may be evaluated in rodents using appropriate behavioral tests. For example, a combination of properly designed tests in the radial maze, Morris water maze, object recognition and object location allows to be evaluated in animal models. Functions such as verbal learning can’t be evaluated in MHE and different mechanisms are involved in the impairment of different components. Also, there are pharmacological treatments that restore selectively working or reference memory and spatial or non-spatial learning and memory in rats with MHE. There are also appropriate test to assess some motor alterations: hypokinesia, fine motor coordination or balance. Studies based on proper use of animal models will accelerate the advance in understanding the mechanisms involved in MHE and will open new therapeutic approaches to improve quality of life and life span of the patients.

METHODS: Male C57Bl/6 mice were treated with azoxymethane (AOM, 100 mg/kg BW) to induce ALF. Six hours after AOM injection, mice were injected with the TSP-1 antagonist LSKL (30 mg/kg) or SLLK as control. In parallel, male TSP-1 knockout mice and wild-type (WT) controls were injected with AOM. In AOM-treated mice, cognitive impairment was monitored by reflex and ataxia measurement. Liver histology was assessed by hematoxylin and eosin staining and serum transaminases were measured. Cleaved caspase 3 immunohistochemistry and TUNEL staining were used to assess apoptosis in liver tissue. Hepatic inflammation was determined by measuring IL-1β, IL-6 and TNFα expression via real-time PCR and ELISA assays. TGFβ1 and TSP-1 expression were assessed in liver, serum and cortex by immunoblotting, caspase 3 immunohistochemistry and TUNEL staining were used to assess apoptosis in liver tissue. Hepatic inflammation was determined by measuring IL-1β, IL-6 and TNFα expression via real-time PCR and ELISA assays. TGFβ1 and TSP-1 expression were assessed in liver, serum and cortex by immunoblotting, immunohistochemistry and real-time PCR. Cerebral edema and microglia activation and strategies employed to reduce TSP-1 signaling reduced liver damage and neuroinflammation was measured by assessing IL-1β, IL-6 and TNFα expression in the cortex. RESULTS: Mice injected with AOM had elevated hepatic, circulating and cortical TGFβ1 and TSP-1 levels, with the cortex only having elevated TGFβ1. LSKL-treated mice and TSP-1 knockout mice administered AOM had reduced activation of hepatic TGFβ1, hepatocyte apoptosis, inflammation, and hepatic injury compared to AOM and SLLK-treated mice or WT AOM-treated mice. LSKL-treated and TSP-1 knockout mice administered AOM had an increased latency to reach coma compared to SLLK-treated or WT mice. LSKL-treated mice and TSP-1 knockout mice had reduced TGFβ1 expression, less cerebral edema, attenuated microglia activation, and decreased expression of IL-1β, IL-6 and TNFα in the cortex compared to control mice. CONCLUSIONS: TGFβ1 and TSP-1 were elevated in the livers and serum of AOM-treated mice and strategies employed to reduce TSP-1 signaling reduced liver damage and neuroinflammation in the AOM mouse model of HE. Therefore, targeting TSP-1 signaling may be a novel therapeutic target for the management of both ALF and HE following acute liver injury.

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Thrombospondin-1 Worsens Azoxymethane-Induced Hepatic Encephalopathy Through Activation of Transforming Growth Factor Beta 1

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BACKGROUND: Acute liver failure (ALF) is a consequence of severe hepatic injury and is associated with poor clinical outcomes. Patients with ALF often present with neurological complications, called hepatic encephalopathy (HE). Transforming growth factor beta 1 (TGFβ1) is upregulated following liver damage and we have shown that TGFβ1 drives HE progression. Thrombospondin-1 (TSP-1) can activate latent TGFβ1 and therefore, we hypothesize that hepatic-derived TGFβ1 is activated by TSP-1, which exacerbates liver damage and HE associated with azoxymethane-induced ALF.

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Prediction of Overt Hepatic Encephalopathy by the Continuous Reaction Time Method and the Postosynaptic Encephalopathy Syndrome Test in Patients With Cirrhosis

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BACKGROUND: Predicting overt hepatic encephalopathy (OHE) is of major importance because the condition is unpleasant, requires hospitalization, and partly preventable. The risk is related to pre-existing discrete cognitive defects, and a number of psychometric tests are validated for detection of such defects. For clinical practice it is recommended to apply two different tests. We used the Continuous Reaction Time test (CRT) and the Porto Systemic Encephalopathy Test (PSE), and examined their single and combined value for prediction of OHE in cirrhosis patients.

METHODS: We studied 130 mentally unimpaired cirrhosis patients by the two tests and followed them up for on average 38.5 months. The CRT measures velocity and stability in motor reaction times to 150 repeated auditory. The PSE is a paper-and-pencil test measuring the duration of completing 5 tasks. We collected data on episodes of OHE during follow-up. The clinical course was analysed in patient groups according to the outcome of each test and of both tests together. No anti-HE treatment was initiated except for OHE.

RESULTS: We observed 74 OHE episodes. The PSE was abnormal in 47 and predicted 31 OHE episodes among 14 patients (PPV = 29%, NPV of 75%, sens. 40%, spec. 65%). The CRT test was abnormal in 74/130 and predicted 54 OHE episodes among 23 patients (PPV = 31%, NPV = 78%, sens. 65%, spec. 46%). One or both tests were abnormal in 87/130 and predicted 60 OHE episodes among 27 patients (PPV = 31%, NPV = 81%, sens. 77%, spec. 40%). 43/130 had two normal tests but 8 experienced OHE and had 14 OHE-admissions.

CONCLUSIONS: The combined use of PSE and CRT test identified 77% of patients who later experienced OHE while ruling out future OHE with an NPV of 81%.

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Characterization of a Novel Mouse Model of Type A Hepatic Encephalopathy

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BACKGROUND: Type A hepatic encephalopathy (HE) describes the neurological complications caused by acute liver failure. Research into Type A HE has been hampered as existing models cause severe and rapid HE, with a limited therapeutic window and high mortality, that do not reflect all features observed in patients. The aim of this study was to establish and characterize an alternative mouse model of Type A HE that has a more protracted timeline of pathology.

METHODS: Male C37Bl6 mice were fed standard rodent chow enriched with 0.1% 3,5-diethoxycarbonyl-1,4-dihydrocollidine and 10% ammonium acetate (DDC + NH4). Neurobehavioral indices and neuromuscular deficits were assessed by open field test, rotarod, grip strength test and gait analysis. Serum, liver and brain tissue were collected after 13 days of DDC + NH4 feeding. Liver damage was assessed by serum chemistry and H&E staining. Serum and cortical ammonia and total bile acid content were assessed with colorimetric assays. Cerebral edema was assessed using the wet weight/dry weight method. Microglia activation was assessed by Iba1 immunohistochemistry. The expression of proinflammatory cytokines were assessed by qPCR and ELISA.

RESULTS: DDC + NH4 feeding caused significant neurological and neuromuscular deficits in every test performed commencing after 3–7 days of feeding. The liver damage observed was like a cholestatic...
Attenuation of Neurological Symptoms of Type C Hepatic Encephalopathy by Selective Ablation of Neuronal FXR Expression

Stephanie Grant1,2, Eainia Williams1,2, Anca Petrescu, PhD1,2, Matthew McMillin, PhD1,2, Gabriel Framepton2,3, Sharon DeMorrow, PhD1,2,3.

BACKGROUND: Hepatic encephalopathy (HE) describes the neurological deficits that result from liver impairment. Liver disease is associated with an increase of circulating bile acids that can cross the blood brain barrier and activate FXR receptors in neurons. We have previously demonstrated that aberrant bile acid signaling via activation of neuronal FXR contributes to HE pathogenesis in rodent models of acute liver failure. However, a role for FXR-mediated bile acid signaling in HE due to chronic liver cirrhosis is undefined.

METHODS: Neuron-specific FXR knock-out mice were generated by crossing Floxed FXR mice with SNAP-25 cre recombinase mice. The resulting mice were designated FXRflu-neu generated by crossing Floxed FXR mice (FXRΔneo) with floxed FXR mice (FXRΔneo). FXRΔneo were treated with carbon tetrachloride (CCl4; 1 ml/kg) by oral gavage twice per week for 12 weeks. Neurobehavioral indices and neuromuscular deficits were assessed by open field test, rotated, grip strength test and gait analysis. After 12 weeks, tissue was collected and liver damage was assessed by serum chemistry and H&E staining. Total bile acid content was assessed in the cortex and cerebellum using colorimetric assays. The expression of ASBT, FXR, and its downstream effectors was assessed by qPCR and immunofluorescence. The expression of proinflammatory cytokines was assessed by qPCR and ELISA. Total, total bile acids, ASBT and FXR expression were assayed in brain tissue from cirrhotic patients with HE, compared to cirrhotics without HE and age- and gender-matched controls that had been collected and banked by the Australian Brain Bank Network.

RESULTS: Total bile acid content was elevated in the cortex and cerebellum in CCl4-treated WT mice and in cirrhotic patients with HE, compared to cirrhotics without HE and non-liver impaired controls. Furthermore, ASBT, FXR and SHP expression were increased in the frontal cortex of mice and humans with HE, but not in non-HE control samples. WT and FXRΔneo mice treated with CCl4 had significant deficits observed in every neurobehavioral and neuromuscular test performed, as well as marked microglia activation and increased proinflammatory cytokine expression compared to vehicle-treated mice. These neurological deficits and neuroinflammation were attenuated in FXRΔneo mice after CCl4 treatment, even though liver damage was comparable in all genotypes of mice used.

CONCLUSIONS: These data indicate that neuronal expression of FXR plays an important role in the development of HE. Specific targeting of FXR activation in the brain may be a potential therapeutic target for the management of HE.
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Animal Naming Test Is Highly Accurate and Reliable for Diagnosis of Minimal Hepatic Encephalopathy in Outpatients With Cirrhosis
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BACKGROUND: Background and Aims: Minimal hepatic encephalopathy (MHE) is the mildest form in the spectrum of hepatic encephalopathy that impairs health-related quality of life. PHES remains the gold standard for the diagnosis of this condition. Animal naming test (ANT) is reliable and sensitive tool for diagnosis of MHE and can also predict overt episodes of HE. We compared usefulness of PHES and ANT for the diagnosis of MHE and for the prediction of the development of overt episodes of HE.

METHODS: Between July 2017 to June 2018, one hundred and three consecutive patients with liver cirrhosis without overt HE were subjected to PHES and ANT evaluation. MHE was diagnosed when the PHESS was ≥ 5. Receiver-operating characteristic (ROC) curve was used to determine the optimum cut-off of ANT value for the diagnosis of MHE. The best specificity and sensitivity was found at <14. Patients were followed up every 3-6 months till October 2018.

RESULTS: Thirty-seven (35.9%) patients had MHE as assessed by altered PHES. ANT (<14) was present in 36 (34.95%) patients with MHE with sensitivity of 89.19% and specificity of 91.75%, PPV of 93.54%, NPV of 94.03% and diagnostic accuracy of 93.20%. The area under the curve for diagnosis of MHE was 0.978 (95% CI 0.954–0.996). MHE patients had significantly lower ANT as compared to non-MHE patients and controls (10.81 ± 0.32 vs 15.27 ± 0.17 vs 15.78 ± 0.19, respectively, P < 0.01). MHE patients had lower hand grip strength compared to non-MHE patients and the control group (Males: 26 vs 30 vs 38, Females 25 vs 28 vs 28, P < 0.05). PHES significantly correlated with Child-Pugh (r = 0.421, P = 0.001) and model for end-stage liver disease (MELD) (r = −0.345, P = 0.001) scores. ANT correlated with PHES (r = 0.752, P < 0.001) and also with Child-Pugh (r = −0.408, P = 0.001) and MELD (r = −0.318, P = 0.001) scores. During follow-up, 14 patients in MHE group and 4 in non-MHE group developed overt episodes of HE (P = 0.001). Out of 37 patients with abnormal PHES 14 patients developed overt HE on follow up and out of 36 patients with abnormal ANT 14 patients developed overt HE on follow up. 33 patients had both PHES and ANT abnormal. 4 patients had PHES abnormal and ANT normal. 3 patients had PHES normal and ANT abnormal.

CONCLUSIONS: ANT is a highly accurate and reliable tool for the diagnosis of MHE and prediction of overt episodes of HE in outpatients of cirrhosis as compared to PHES and correlates well with the Child-Pugh and MELD scores.

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Multimodal Approach Including MR-spectroscopy for the Diagnosis of Minimal Hepatic Encephalopathy
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BACKGROUND: There is no gold-standard for the diagnosis of hepatic encephalopathy (HE) and especially in case of minimal HE (MHE) where the value of paraclinical examinations is debated. MR-spectroscopy has been proposed as a valuable diagnostic tool for the diagnosis of HE by showing increased glutamate/glutamine peak and decreased myoinositol and choline peaks. However, access to MRI is difficult and few data on real life experience has been reported. We studied the interest of a multimodal approach combining clinical, neuropsychological, and MR-spectroscopy in the diagnosis of MHE.

METHODS: We conducted a retrospective study in a single tertiary university hospital in Paris, France, where all out-patients referred to a specific hepatology-neurology consultation dedicated to the diagnostic of MHE underwent a clinical examination, psychometric tests (Critical Flicker Frequency-PHES, Psychometric Hepatic Encephalopathy Score-PHES), ammonemia and cerebral MRI with spectroscopy. Patients with a previously unreported decrease in the neurotransmitters glutamate, GABA and N-acetylaspartate. No statistically significant differences were observed between the CLD patients and controls.

CONCLUSIONS: In patients with compensated CLD, there were no significant neuro-metabolic alterations as assessed by high resolution 1H-MRS. In CPSS, however, neuro-metabolic changes were clear, and likely related to measurably impaired neurocognitive functioning. Together, these results suggest that in CPSS (type B encephalopathy) the brain is likely exposed to a higher load of neurotoxic substances than in patients who have some degree of portal flow (type C).

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Drug-induced Hyperammonemia: Data From Vigibase, the WHO Database
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1Brain Liver Pitié-Salpêtrière Study Group (BLIPS), Sorbonne Université, Groupe Hospitalier Pitié-Salpêtrière Charles Foix, INSERM UMR S_938/CDDR Saint-Antoine & Institute of Cardiometabolism and Nutrition (ICAN), Paris, France; 2Université Paris Est Créteil, Service d’Hépatologie, Créteil, France.

BACKGROUND: Altered consciousness secondary to metabolic encephalopathies represents a major cause of ICU admission with favorable outcome when diagnosed and treated rapidly. Ammonia dosage is thus recommended in most textbooks in the absence of any diagnosis after etiological work-up encompassing biological sampling, cerebral imaging and EEG. Despite hyper-ammoniaemia is most commonly secondary to liver diseases, portosystemic shunts, inborn errors of metabolism, most commonly cycle defects, microbial pullulation or drug-induced hyperammonemia (DIH) are other possible causes. DIH is poorly described but is mainly recognized as the consequence of valproic acid. Some antiepileptic agents, fluoroauracil or aspiraginase, have been implicated but this class is evoking rapidly. To describe the drugs associated with DIH.

METHODS: We used Vigibase, the WHO global Individual Case Safety Report (ICSR) database, which contains reports of suspected adverse drug reactions (ADRs) collected by national drug authorities in over 130 countries between 1967 and 8 May 2019. This observational retrospective study included all ADRs reported as ‘hyperammonemia’ according to the Medical Dictionary for Drug Regulatory Activities (MedDRA)v21.1 term (Preferred term (PT) level). The drugs considered in the analysis were those notified as suspected treatments. Drugs used to treat hyper-ammoniaemia or hepatic encephalopathy were excluded as were drugs reported less than 3 times. Drugs with a positive lower end of the 95% credibility interval for the information component (ICO25) > 0. An indicator value for disproportionate Bayesian reporting, was considered as causative of hyperammonemia.

RESULTS: Among 19 438 165 ICERS, 576 drugs were identified for the term ‘hyperammonemia’ (PT). Six were excluded because they were used to treat hyperammonemia or hepatic encephalopathy. Thus, 29 drugs had an ICERS > 0 and represented 2792 cases (0.014%). Twelve drugs were reported more than thirty times (Table 1).

CONCLUSION: Besides commonly involved drugs, some other commonly used drugs seem associated in DIH. These data could help in the etiological work-up of hyperammonemia.

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Increased Levels of Xerobiotics in Plasma of Cirrhotic Patients With Neurological Symptoms, a Metabolic Study
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BACKGROUND: Encephalopathy is a classical complication of liver disease and/or portosystemic shunts. Its pathophysiology is not completely elucidated; mechanisms include the role of elevated ammonia levels in association with systemic inflammation. An impairment of blood-brain barrier (BBB) permeability is also hypothesized. Metabolomics enables to detect a wide range of metabolites without any a priori. In a recent metabolomic study including patients who underwent cerebrospinal fluid (CSF) collection, our group outlined that xenobiotics/drugs that usually are not able to cross BBB were retrieved in the CSF, suggesting a potential neurological toxicity of drugs. CSF collection is invasive. To describe the xenobiotics present in the plasma of cirrhotic patients, using the same metabolic approach.

METHOD: We conducted a retrospective study of plasma samples in the Hepatological ICU. Plasma samples from cirrhotic patients displaying encephalopathy were compared to plasma from cirrhotic patients without neurological symptoms, and to plasma from healthy controls. Liquid chromatography-coupled to high-resolution mass spectrometry was performed and thereafter the metabolic fingerprints were compared to database and between the different groups.

RESULTS: Plasma samples were obtained from 12 cirrhotic patients with encephalopathy (age 59 [40–68], MELD 20 [16–31], alcohol 58%), 13 cirrhotic patients without encephalopathy (age 56 [55–64], MELD 17 [14–29], alcohol 38%) and 9 healthy controls. Among 495 identified metabolites, ammonium > 50 μmol/l, MBI T1 hyperintensity of the basal ganglia and an MRS HE profile sug- gestive of HE were all statistically associated with the diagnosis of MHE (P < 0.0001). The best diagnostic performance was achieved by combining MRS with either MBI T1 hyperintensity (AUC = 0.93) or ammonium (AUC = 0.91).

CONCLUSION: A multimodal approach combining clinical data, ammonium and cerebral MBI with MRS seems to have good accuracy for the diagnosis of MHE. Further prospective studies are mandatory.
25 corresponded to xenobiotics or its derivatives. Fluoxetine was detected with a more than 200 fold increase, ammonia with a more than 10 fold increase and benzyl alcohol (present in cough pills and antiseptics) with a 3 fold increase in cirrhotic patients with encephalopathy as compared to cirrhotic patients. In cirrhotic patients with or without encephalopathy, propranolol was detected with a more than 8500 fold increase, acetaminophen with a 40 fold increase, penicillamine and ampicillin with a 2 fold increase as compared to healthy controls. Interestingly, several substances which were not expected to have systemic diffusion were detected in cirrhotic patients and in healthy controls: eugenol, isoeugenol (used in mouth bathing solution), triethanolamine (trolamine, used in cutaneous creams) and resorcinol monoacetate (used in mouth bathing solution and in cutaneous creams).

CONCLUSION: Cirrhotic patients, especially those with neurological symptoms, display dramatically increased levels of several xenobiotics in plasma. These results confirm that PK/PD parameters of commonly used drugs are highly modified in these patients. This suggests a potential role of xenobiotics in the pathophysiology of encephalopathy in patients with liver diseases.

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Hippocampal and Cerebellar Astrocytes Morphological Alterations in a Rat Model of Chronic Hepatic Encephalopathy

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BACKGROUND: Chronic hepatic encephalopathy (CHE) is a spectrum of neuropsychiatric abnormalities in patients with chronic liver disease. The hippocampus and cerebellum are key regions implicated in the cognitive and fine motor deficits of CHE. Astrocytes can sense neuronal activity through neurotransmitter-receptors and ion-channels, modulate the neural circuits and control energy homeostasis through morphological plasticity. They adjust their volume by releasing osmolytes through neurotransmitter-receptors and ion-channels, modulate the neural circuits and control energy homeostasis through morphological plasticity.

RESULTS: The American Journal of GASTROENTEROLOGY

**Table 1:**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Number of Processes/Region</th>
<th>Significant Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valproic acid</td>
<td>221</td>
<td>14.5%</td>
</tr>
<tr>
<td>Fluorouracil</td>
<td>96</td>
<td>63.8%</td>
</tr>
<tr>
<td>Topiramate</td>
<td>55</td>
<td>18.1%</td>
</tr>
<tr>
<td>Oxaliplatin</td>
<td>74</td>
<td>26.2%</td>
</tr>
<tr>
<td>Asparaginase</td>
<td>71</td>
<td>39.5%</td>
</tr>
<tr>
<td>Levofolic acid</td>
<td>54</td>
<td>37.2%</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>32</td>
<td>20%</td>
</tr>
<tr>
<td>Ticlopride</td>
<td>18</td>
<td>38.4%</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>34</td>
<td>32.2%</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>32</td>
<td>19%</td>
</tr>
<tr>
<td>Methotractol</td>
<td>33</td>
<td>41.1%</td>
</tr>
</tbody>
</table>

*Increasing number of processes is evidenced by an increase in GFAP expression. Overlapping GFAP and DAPI indicates an activation of astrocytes.*

CONCLUSIONS: To our knowledge, this is the first report showing significant alterations in astrocytes count and important morphological changes already 4-weeks post-BDL in the hippocampus and cerebellum. Increase in GFAP+ cells may be related to the stimulation of mature astrocytes and reentering into proliferation cycle which is similar to proliferating neonatal astrocytes and is a common situation in various neuropathological disorders. The proliferation of activated glial-cells can induce additional inflammatory reactions and creation of gli-scar, something to be investigated in future studies. Finally, the astrocytosis and astrocyte morphology changes may alter the CNS microenvironment that usually ensures neuronal health and may contribute to the cognitive impairment of BDL rats.

REFERENCES:

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Brain Regional Susceptibility to Oxidative Stress in a Rat Model of Chronic Hepatic Encephalopathy: In Vivo 1H MRS, Ex Vivo ESR Spectroscopy and Histology Findings

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BACKGROUND: Oxidative stress (OS) is believed to be an important feature in the pathogenesis of chronic hepatic encephalopathy (CHE). In CHE impaired ammonium clearance by the diseased liver leads to brain glutamine accumulation. In-vitro, affected ammonium detoxification together
A) Hippocampus - dentate gyrus

B) Hippocampus - hilar

C) Sholl analysis

D) Hippocampus - hilus

Cerebellum - granular layer

Fig. 1  A) Representative micrographs of double staining for anti-GFAP (red) and DAPI (blue) of the hippocampus of sham, BDL w4 and BDL w8 post-op rats, scale bar: 500 μm. B) Astrocytes density quantification at the hippocampus hilus and cerebellum granular layer. Note the increase in astrocytes number at week 4 post BDL, two-way-ANOVA: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, mean±SD. C) Astrocytes Sholl analysis – morphological characterization of the number of intersections of processes branches with radii at various distances from the cell body. D) Representative micrographs of brain sections from sham and BDLs rats at 4 and 8 weeks post-op stained with anti-GFAP (red) and DAPI (blue), scale bar: 25μm. E) Sholl analysisof GFAP-labelled astrocytic intermediate filaments (IMF) showed a significant time dependent decrease of the number of processes, intersections (decrease of intersections in each of the three concentric rings) and the mean length of the IMF observable within the section, two-way-ANOVA:*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, mean±SD.
A) *In-vivo* 'H-MRS – antioxidants detection as indirect OS sign

- Hippocampus
  - Scan 0 hippocampus
  - Gln
  - Asc
  - GSH

- Cerebellum
  - Scan 0 cerebellum
  - Gln
  - Asc
  - GSH

B) *EX-vivo* ESR – direct OS detection

- ESR Silent
  - CO₂
  - OH
  - COCH₂

- ESR Active
  - H₂O₂

- **Fig. 1 A)** Evolution of Gln, Asc and GSH, from week 0 (before BDL) to week 8 post-BDL. **B)** Cell permeable-non-toxic spin probe for the quantification of extra and intracellular $O_2^\cdot$ production and detection of ROS. The spin adduct is resistant to reduction by vitamin C (Asc) and thiols (GSH), therefore allows to quantitative detection of $O_2^\cdot$. ROS generation rate is calculated from ESR kinetics plots. **C)** Photomicrographs of histological sections of cerebellum – immunohistochemical staining of GPX1 on 10µm sections showed increased immunoreactivities in Purkinje and granular cells layer of BDL rat (arrowhead – Purkinje cells layer, arrow – granular cells layer, * - molecular layer and WM – white matter). Purkinje cells of BDL rat revealed shrinking soma having the mean size of 8.04±1.8µm² while the size of Sham is 12.5±1.2µm² (p<0.00001). Two way-ANOVA: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, mean±SD.
with glutamine induces reactive-oxygen-species (ROS) generation associated with astrocyte impairment.2,3 ROS play an important role in cellular signalling, synaptic-plasticity, learning and memory. When in excess, they cause cellular damage.4,5 Systemic oxidative stress was previously shown in bile-duct-ligated rats (BDL). Using in-vivo-longitudinal 1H-MRS we previously observed the indirect presence of OS as a decrease of brain Asc in the hippocampus and cerebellum of BDL rats (model of CHE).6 We aimed to validate these findings using for the first time ex-vivo electron spin (ESR) spectroscopy and histological measures to assess OS levels.

**METHODS:** In vivo-1H-MRS: Cerebellum/hippocampus of adult rats were scanned before BDL and after every 2-weeks up to 6 weeks (n = 18) at 9.4T-MRI (Varian/MagnetiScience) using SPECIAL-sequence (TE = 2.8 ms). Ex-vivo-ESR: ESR 300E (Bruker-BioSpin) was used for intracellular superoxide anion detection. Hippocampus/cerebellum were extracted at 6 weeks post-surgery (n = 5). After homogenization, intracellular superoxide anion was determined using NBT/Sham-surgery (n = 5). Cell viability was assessed with MTT assay. Histological staining with GPX1-enzyme revealed an increase in cerebellum suggests that GSH-synthesis may increase (conversion of GSH to GSSG). OS is involved in the propagation of cellular injury and may be an important factor in the etiology of the CHE.

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**Quality of Life Assessment May Aid in the Diagnosis of Minimal Hepatic Encephalopathy and Prediction of Overt Hepatic Encephalopathy**

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**BACKGROUND:** Minimal hepatic encephalopathy (MHE) impinges on quality of life (QoL), is associated with a high risk of overt hepatic encephalopathy (OHE) and is often treatable by simple means. Still, MHE is rarely systematically diagnosed and treated likely because dedicated psychometric tests give the impression of being resource heavy and thus scare off many clinicians. Simple, patient-administered QoL questionnaires e.g. sickness impact profile (SIP), could improve diagnostic rates. This approach was tested in a US-based study introducing SIP/CH2 score (formula of 4 SIP statements, gender and age). We here aim at externally validating SIP/CH2 score in a cirrhotic cohort using continuous reaction-time (CRT) test and portosystemic hepatic encephalopathy score (PHS) for MHE diagnosis.

**METHODS:** 110 cirrhotic patients without OHE (age 60 years, MELD 11.4, 80% blue-collar) completed cognitive testing and SIP. Abnormal CRT and/or PHS diagnosed MHE. SIP consists of 136 questions inquiring about QoL and standardized QoL scores were compared in MHE and non-MHE patients. The SIP/CH2 (US cut point) was applied as a predictive values were calculated. We followed the patients for 2.7 years on average and registered OHE episodes.

**RESULTS:** The SIP/CH2 was abnormal in 82/110 patients and was in agreement with the psychometric tests in 73/110 cases (66%). The SIP/CH2 indicated MHE in 58/71 of the patients with MHE according to 2 MHE psychometric (positive predictive value = 71%, sensitivity = 82%, AUTROC 0.63). The SIP/CH2 was false positive in 24/39 non-MHE patients (positive predictive value 38%, NPV 53%). A normal SIP/CH2 did not exclude MHE in our population as 13/28 (46%) with a normal SIP/CH2 score had MHE according to the CRT and PSE tests. In our cohort using a cut point of ≥40, we obtained, >0.49, slightly improved correct classification to 72% of patients. Only 42% (14%) with a normal baseline SIP/CH2 experienced OHE, while 29% (30%) with abnormal SIP/CH2 experienced OHE (P = 0.05). Accordingly, the SIP/CH2 positive predictive value for a future HE episode was this 87% (likelihood ratio 2.4).

**CONCLUSIONS:** In conclusion, the idea of a patient-reported outcome score as an addition to standard psychometric is appealing. The US derived SIP/CH2 score is able to identify the majority of patients with MHE and future OHE episodes, but lacks diagnostic specificity. We speculate that development of regional SIP/CH2 scores could be useful. In future studies using the SIP/CH2 as a measure of patients-experienced effect of MHE treatment would be of interest.

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**Facial Cyanotic Profiling Provides Novel Insights Into Intestinal Barrier Disruption and Bacterial Translocation in Acute Decompensation of Cirrhosis**

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**BACKGROUND:** Intestinal dysbiosis with gut barrier impairment (GBI) and bacterial translocation (BT) are recognised as central features of acutely decompensated cirrhosis (AD) and propagating hepatic encephalopathy (HE). Gut mucosal function is technically challenging to investigate, with a difficulty in obtaining representative tissue in cicatrictis and a paucity of non-invasive techniques. A novel assay we developed was to quantify facial cyanoses (FC) as surrogate markers of intestinal inflammation, compared to GBI markers and systemic cytokines.

**METHODS:** Protein was extracted from faeces of patients with stable cirrhosis (SC; n = 50), MELD ≥ 16, and healthy participants (HC, n = 31). A multiplex panel of 13 cyanoses were quantified by electrochemiluminescence or ELISA in paired faecal lysates and plasma. Intestinal epithelium-associated fatty acid binding protein 2 (FABP2) and intestinal-microbiota metabolite D-lactate, as markers of intestinal barrier disruption, were quantified by electrochemiluminescence (ECL) or ELISA in paired faecal lysates and plasma. Faecal FABP2 and D-lactate (P = 0.0011 for both) and intestinal-microbiota metabolite D-lactate, as markers of intestinal barrier disruption, were quantified by ECL or ELISA in paired faecal lysates and plasma.

**RESULTS:** Median ages and MELD scores of AD vs SC patients were 61 (53-68) vs 55 (44–59) years and 18 (13–26) vs 7 (7.0–8.5), respectively. Male gender predominated (AD: 65%, SC: 77%). The causes of cirrhosis were alcohol, NAFLD, and treated hepatitis C. Median venous a mechanical liver is significantly higher in AD vs SC (51 [38–74] vs 26 [36–59], P = 0.0027). Facial FC 18-23 was significantly elevated in AD vs HC (P < 0.0007), important in propagating pathobiology Th17 cell effector function. Facial FC and D-lactate (P = 0.0001 for both) and plasma FABP2 and D-lactate (P < 0.0001) were significantly elevated in AD vs SC and AD vs HC, respectively, consistent with GBI. FABP2 was the faecal marker that correlated most strongly with disease scores (Spearman’s rho: FC=0.446, P < 0.0001; MELD 0.488, P < 0.0001). With the exception of D-lactate and FABP2, FC and D-lactate were more discriminant than matching equivalent molecules in discriminating AD from SC by Principal Component Analysis (Figure 1).

**CONCLUSIONS:** FAC profiling represents a novel targeted approach to localised measurement of the intestinal cytokine milieu in cirrhosis, and in conjunction with D-lactate, demonstrates that pro-inflammatory AD from SC may be distinguished by integrating FC and D-lactate. The AD gut microbiome environment may skew T cell priming away from restorative Thg and towards inflammasome Th17 profiles, promoting epithelial barrier damage and preventing repair. These data provide insights into intestinal mucosal injury and GBI, as a novel pathobiology in AD and HE.
Acetate Attenuates the Astrocyte Swelling and Brain Edema in Severe Liver Failure

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BACKGROUND: Astrocyte swelling and brain edema are major complications of severe liver failure. Ammonia plays a major role in the development of astrocyte swelling/brain edema in this condition. However, current therapies have not thus far improved the outcome of liver failure induced astrocyte swelling/brain edema. Since acetate has been shown to have neuroprotective effect in other neurological conditions, likely though increased energy production, we examined whether acetate similarly protects cell swelling in cultured astrocytes post-ammonia exposure. We also examined whether treatment of rats with glicyrrhetacate (GTA), an acetate precursor, is known to increase circulating, as well as tissue levels of acetate, alleviates the brain edema induced by the liver toxin thiacetamide (TAA).

METHODS: Astrocyte cell volume was estimated by measuring the intracellular water space using 3-O-methylglucose. Brain water content was measured by using the gravimetric method.

RESULTS: Exposure of astrocyte cultures to pathological concentrations of ammonia (NH4Cl, 5 mM) for 24 h significantly increased cell swelling. Co-treatment of ammonia with acetate reduced such swelling in a dose-dependent manner. Further, treatment of rats with TAA (250 mg/kg bw) for 3 days increased the brain water content, and that pretreatment of (intragastrically) TAA-treated rats with GTA (7.5 g/kg bw), attenuated brain edema.

CONCLUSIONS: These findings strongly suggest that acetate supplementation will exert salutary effects in reducing brain edema in patients with severe liver failure.

Cholestasis Decreases Dendritic Spine Density in a Rat Hippocampal Organotypic Culture

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BACKGROUND: Executive functioning impairment in children with cholestatic liver disease is increasingly recognized. Injury to developing neuronal networks could be an underlying mechanism. Given that the CSF of rat pups following bile duct ligation is highly concentrated in ammonia, mur- ocholic and taurocholic acids, we hypothesized that cholestasis may significantly affect density of synaptic contacts onto pyramidal neurons, known to be essential in the development of executive functioning.

METHODS: Transverse hippocampal organotypic slice (400 µm thick) were harvested from 6–7-day-old Wistar rats. They were maintained for 15 days in a CO2-incubator (33°C). pc-DNA3.1-EF1FP plasmid bidirectional transfection was performed 7 days after harvesting. 3 days following transfection, control medium or experimental medium containing 100 µM o-muricholic-acid, 200 µM taurocholic acid and 2.5 mM ammonium was added to the culture (day 0 of exposure, MIX condition). Confocal microscopy using imaged was used for to manually count CA1 pyramidal neuron dendritic spines as proxies for excitatory synapses.

Static analysis quantified dendritic spines density each day. Dynamic analysis quantified dendritic spines turnover (loss and neo-formation) for each 24 h time-window. Statistical analysis was conducted using PRISM software for multiple t-test or mixed-effect ANOVA.

RESULTS: Static analysis showed a biphasic profile in MIX condition. During early phase (first 3 days of exposure), we observed >50% decrease in dendritic spine density compared to control (cf Figure 1, P < 0.001). On days 3 to 4, spine density recovered to reach control value. Dynamic analysis showed 15% loss in dendritic spines stability during the early phase of exposure to MIX condition, compared to controls, with comparable low rates of spine turnover. During the late phase of MIX exposure, spine turnover increased significantly in favor of spine neo-formation: spine neo-formation was 10 times higher (0.280 vs 0.023 spines µm-1, P < 0.0033) than controls, while spine loss was 6 times higher in neurons exposed to MIX (0.154 vs 0.026 spines µm-1, P = 0.00266) than controls.

CONCLUSIONS: We demonstrate here that mimicking cholestasis ex vivo leads to a biphasic response in spine density of rat hippocampal CA1 pyramidal neurons. Spine density decreases during...
Reversible and Irreversible Neurological Complications in Hepatic Encephalopathy
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BACKGROUND: Hepatic encephalopathy (HE) is a major neurological condition that occurs due to acute and chronic liver failure following drug toxicity, viral hepatitis, or exposure to various hepatotoxins. Acute HE (Type A HE) is associated with cerebral edema, increased intracranial pressure, coma and death. Chronic HE (Type C HE) is characterized by mental confusion, behavioral changes, and motor disturbances. It is currently unclear whether HE is reversible or irreversible. We therefore examined whether acute or chronic liver failure is reversible or irreversible in the thioacetamide (TAA) rat model of acute and chronic HE. Accordingly, rats were treated with the liver toxin TAA and brain edema, neurobehavioral, cognitive and motor deficits were measured.

METHODS: Rats were treated with the liver toxin TAA and brain edema, neurobehavioral, cognitive and motor deficits were measured.

RESULTS: Treatment of rats with high doses of TAA (to induce acute HE) led to brain edema within 2-3 days. Rats following TAA treatment were examined for longer time periods. Rats did not exhibit any brain edema, although they expressed neurobehavioral, cognitive and motor deficits without any changes documented in blood and brain ammonia levels, as well as liver failure markers, as compared to normal rats. However, rats that underwent chronic liver failure for 10 days displayed neurobehavioral, cognitive and motor deficits, along with brain structural and molecular events, including reduced levels of astrocytic matrix molecules, as well as reduced levels of neuronal proteins. We also found a reduction in synaptic density and in dendritic complexity. These changes correlated well with increased blood/brain ammonia levels and with liver failure markers. However, when TAA treatment was withdrawn (after 10 days), and the rats monitored for longer time periods still showed neurobehavioral, cognitive and motor deficits, as well as defective neuronal integrity, even though blood and brain ammonia levels, as well as liver biochemical and molecular parameters were reversed.

CONCLUSIONS: These findings strongly suggest that neurobehavioral, cognitive and motor deficits in HE cannot be reversed even when acute or chronic liver failure had been corrected.

Support: VA Merit review; AASLD/ALF.

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The Contribution of the Blood-Brain Barrier and Choroid Plexus to the Pathology of Hepatic Encephalopathy
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ABSTRACT: Cerebral edema and associated increase in intracranial pressure, along with brain herniation are the major complications found in patients with acute liver failure (Type A HE or acute hepatic encephalopathy, AHE) which represents the most frequent cause of death in these patients. The preponderance of experimental evidence favors a cytotoxic mechanism, and the only cell in brain that undergoes such swelling are astrocytes. While there is no evidence of blood-brain barrier break-down in patients or in experimental animals with AHE, it is unclear how a sustained astrocyte swelling (cytotoxic brain edema) occurring in AHE, ultimately results in increased intracranial pressure, brain herniation and subsequent coma and death in these patients. A major brain component that may be involved in the edema development is the choroid plexus, since epithelial cells of the choroid plexus produce cerebrospinal fluid. Under CNS pathological conditions, the barrier function of blood-CSF barrier is altered, along with changes in the ependyma, leading to a seepage of fluid out of the ventricular system, ultimately resulting in brain edema, along with the entry of inflammatory cells into the brain parenchyma. In preliminary studies, we found activated mast cells in the choroid plexus of the liver toxin thioacetamide (TAA)-treated rats, and such activation significantly increased cytokine production, histamine release and decreased levels of growth factors including platelet derived growth factor, insulin-like growth factor, fibroblast growth factor. We also found increased levels of the water channel proteins aquaporin 1 and 4 (AQP1/AQP-4) in TAA-treated rat brain choroid plexus, and increased brain edema, while inhibition of mast cell activation diminished both AQP levels, as well as brain edema development. These findings strongly suggest that acute liver failure stimulates the activation of choroid plexus mast cells and the subsequent inflammation and AQP1/4 expression, which ultimately contributes to the sustained cytotoxic brain edema found in AHE. We anticipate that studies aimed at a better understanding of the role of choroid plexus inflammatory events in the development of brain edema associated with AHE, will greatly facilitate the identification of agents capable of ameliorating this debilitating condition.

Support: VA Merit review; AASLD/ALF.

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Mechanism of Alzheimer Type II Astrocyte Development: Implication for the Defective Neuronal Integrity and Neurobehavioral Deficits Associated With Chronic Hepatic Encephalopathy
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BACKGROUND: Chronic hepatic encephalopathy (CHE) is a major neurological condition that occurs following chronic liver failure (CLF) following drug-induced hepatotoxicity, viral hepatitis, or exposure to various hepatotoxins. CHE is characterized by mental confusion, behavioral changes, and motor disturbances. The molecular basis for CHE remains elusive. The presence of Alzheimer type II...
astrogliosis (AT2A) is the only histopathological findings observed in CHE. However, little is currently known regarding its development, and its involvement in the pathogenesis of CHE.

METHODS: Astrogliosis maturation factor and other inflammatory factors were measured by western blots, immunohistochemistry, and by ELISA. AT2A was analyzed by histopathology.

RESULTS: We identified increased levels of astrocytic glia maturation factor (GMF), a factor strongly implicated in neuroinflammation and in the overexpression of various inflammatory factors (IL-1β, TNF-α, IL-6, chemokine (C-C motif) ligand 1 (CXCL1), matrix metalloproteinase-3 (MMP-3), pro-angiotensin II (PFGII) and cyclooxygenase 2 (COX2), as well as reduced levels of α-tubulin and glial fibrillary acidic protein, along with increased levels of aggregated protein granular a-synuclein in the thioacetamide-induced rat model of CHE. Further, we identified reduced levels of neuronal proteins, PSD95, synaptophysin, and NMDA-nr1. Moreover, synaptic density and dendritic complexity are reduced post-CLF. Since elevated blood brain ammonia levels have been strongly implicated in the pathogenesis of CHE, while exposure of cultured astrocytes to ammonia was shown to develop AT2A, we utilized this in vitro system to delineate mechanisms by which ammonia contributes to the development of AT2A. We found increased levels of GMF, aggregated nuclear protein lamin a/c, and inflammatory factors (IL-1β, TNF-α, IL-6, CXCL1, MMP-3, PFGII and COX2), as well as reduced levels and oxidized forms of α-tubulin in astrocytes post-ammonia treatment, which was similar to that found in vivo. Further, exposure of cultured neurons to conditioned media (CM) from ammonia-treated astrocytes (AT2A), but not ammonia per se, resulted in reduced levels of neuronal PSD95, synaptophysin and NMDA-nr1, as well as reduced synaptic density and dendritic complexity. Note-worthy, pharmacological inhibition of GMF, or silencing GMF by CRISPR reversed the defective neuronal integrity post-exposure of neurons to CM from ammonia-treated astrocytes in vitro.

CONCLUSIONS: These findings strongly suggest that increased levels of GMF post-CLF may negatively impact neuronal integrity that may ultimately contribute to the neurobehavioral/cognitive and motor deteriora tion observed in CHE. We anticipate that our studies aimed at a better understanding of the molecular mechanisms involved in the development of AT2A, and its impact on neuronal integrity in CHE, will greatly facilitate the identification of agents capable of ameliorating this debilitating condition.

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Central Inhibition of IGFBP3 Attenuates Symptoms of Hepatic Encephalopathy in a Mouse Model of Acute Liver Failure

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BACKGROUND: Hepatic encephalopathy (HE) is a common complication of cirrhosis and negatively affects quality of life and prognosis. However, the development of HE is not inevitable, even in patients with severe hepatic decompensation. Recently, there has been interesting the possibility that the susceptibility to develop HE is, at least in part, genetically determined. In 2017, Cil-Gómez et al. (1) published a study in which 1380 participants from the STOPAH Cohort

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Previously Identified Candidate Gene Associations in Hepatic Encephalopathy Do Not Replicate in the STOPAH Cohort

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BACKGROUND: Hepatic encephalopathy (HE) is a common complication of cirrhosis and negatively affects quality of life and prognosis. However, the development of HE is not inevitable, even in patients with severe hepatic decompensation. Recently, there has been interesting the possibility that the susceptibility to develop HE is, at least in part, genetically determined. In 2017, Cil-Gómez et al. (1) published a study in which 1380 participants from the STOPAH Cohort

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Optimizing the Performance of the Psychometric Hepatic Encephalopathy Score (PHES) for the Diagnosis of Hepatic Encephalopathy

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BACKGROUND: PHES is the most established test for the diagnosis of hepatic encephalopathy (HE). The availability of normative data to adjust for potential confounding variables is a necessary. However, there are other variables which may confound the performance of the PHES test and might result in the misclassification of patients, including: (i) the confounding variables used for test correction, (ii) possible interactions between the corrections variables; (iii) the scoring of the line tracing test (LTT) and (iv) the final summation of the component scores. The aim of this study was to produce a series of models to determine the influence of these variables on the performance of the PHES test.

METHODS: PHES testing was undertaken in 324 permanent UK residents aged > 18 years, who spoke English, and were generally healthy. A UK PHES model was constructed as follows (i) a reciprocal model for the LTT time/err relationship was devised; (ii) univariate/multivariate analyses identified significant correction factors for the individual test scores, without applying interaction terms; and (iii) each corrected test score was transformed to a Z-Score and then summed. The final summed test score was expressed as a Z-Score, values ~< -2.0 were considered abnormal. The effects of changing the basic PHES model by adding in interaction terms, changing the LTT scoring system and the final summation method were examined by applying the models to PHES data collected in 226 patients with cirrhosis.

RESULTS: Seventy-six (36.8%) of the 226 patients had abnormal UK PHES scores. Variation in the scoring of the LTT test (German, Spanish and Italian versions) resulted in a minor reduction in the number of abnormal results (73 [32%]). Add-in and subtraction models for the independent correction factors - age, sex, ethnicity, alcohol consumption, years/place of education - were associated with changes of -11% to +9.8% and ±2.4 to ±8.8 respectively, in the proportion of abnormal result; ethnicity and age had the greatest effects. Adding interaction terms for the correction factors marginally increased the number of abnormal results to 84 (37.2%). Changing the final score summation from Z-scores to integerized scores significantly increased the number of abnormal tests to 100 (44.2%) P = 0.026.

CONCLUSIONS: The major factor affecting the proportion of abnormal PHES results is the method used for final summation of the scores. Interpretation of the scores does not allow for the exact application of thresholds and its use should perhaps be reconsidered.

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Genetically Engineered E. coli Nissle Attenuates Hyperammonemia and Improves Memory in an Experimental Model of Cirrhosis and Hepatic Encephalopathy

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BACKGROUND: Hyperammonemia associated with chronic liver disease (CLD) is implicated in the pathogenesis of hepatic encephalopathy (HE). The gut is a major source of ammonia production that contributes to systemic hyperammonemia in CLD and HE and remains the primary therapeutic target for lowering circulating ammonia. As a therapeutic strategy, Escherichia coli Nissle 1917 bacterium (Ecn), a well characterized probiotic, was genetically modified to consume and convert ammonia to arginine (SYNARG), and its administration to thioacetamide-treated mice resulted in a significant reduction of ammonia levels. SYNARG was further modified to synthesize butyrate
Obesity Accelerates and Exacerbates Neurological Impairments Associated to Hepatic Encephalopathy in Chronic Liver Disease

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AIM: Investigate the synergistic effect of obesity and CLD on the development of neurological impairment in a novel rat model of cirrhosis and obesity.

METHODS: Animal model of CLD and HE. 5-week bile duct ligation (BDL) rats and Sham-operated controls, were used. Groups: Obese-BDL and Obese-Sham received high-fat diet (HFD) for 22-days pre-BDL and high-carbohydrate diet (HCD) for 5-weeks post-BDL. Lean-BDL and Lean-Sham received regular-diet (RD) pre-BDL and HCD post-BDL. Body-weight and fat-mass (EchoMRI) were monitored pre-BDL as well as 3- and 5-weeks post-BDL. Behavior: Motor-coordination, motor-skill learning, and muscular-strength were assessed at 3- and 5-weeks post-BDL. Locomotion and anxiety were measured at 5-weeks. Plasma ammonia, liver enzymes, and lipids were measured at 3- and 5-weeks.

RESULTS: Before BDL surgery, body-weight and fat-mass of rats on HFD increased compared to rats on RD. 5-week post-BDL, body-weight and fat-mass decreased in Lean-BDL and Obese-BDL vs respective Shams, while AST and ALP were impaired in Obese- and Lean-BDL vs respective Shams at 3- and 5-weeks.

BACKGROUND: Hepatic encephalopathy (HE) is a neuropsychiatric syndrome observed in chronic liver disease (CLD/cirrhosis). With an increasing prevalence of obesity-induced cirrhosis and evidence linking blood-derived lipids to neurological impairment, we hypothesize that obesity increases the risk, severity and progression of HE.

RESULTS: Obese-cirrhotic rats developed earlier and was more severe versus Lean-BDL rats. Besides, some neurological impairments developed in Obese-BDL but not in Lean-BDL. These results suggest a synergistic effect, which accelerates/worsens the disease-associated abnormalities in CLD and HE.

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