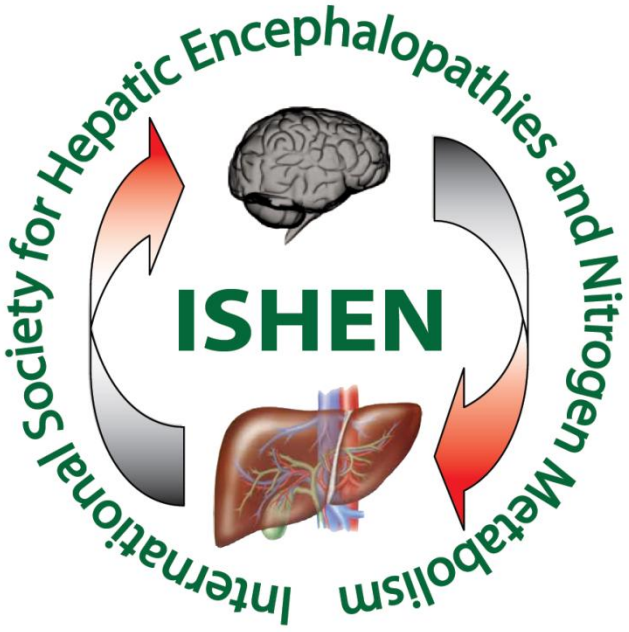

15th ISHEN Symposium

Grenaa, Denmark

May 29 – June 2, 2012

PROGRAM

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER

AASLD

The European Board of Gastroenterology & Hepatology

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GENERAL INFORMATION

VENUE

Kystvejens Hotel og Konferencecenter A/S

Kystvej 26
DK-8500 Grenaa
Denmark

Tel +45 8959 5959

REGISTRATION

Location: Lobby

ISHEN hotline: +45 2125 0444

CERTIFICATE OF ATTENDANCE

Certificates of Attendance are available for all participants upon request at the registration desk.

CME ACCREDITATION

15th ISHEN Symposium has applied for 18 CME credits.

Please enquire at the registration desk about the procedure for obtaining a certificate.

INTERNET ACCESS

Wireless internet is available throughout the venue including the meeting rooms, guest rooms and the lobby. An internet café with printer facilities is also located at the venue.

WIFI connection
Name: kursist
Password: Education

LANGUAGE

The official language of the 15th ISHEN Symposium is English. Simultaneous translation will not be provided.

BUSINESS MEETINGS

Executive Committee Meeting
Thursday May 31 from 12:00 to 14:00 (with lunch).

Society Meeting
Friday June 1 from 18:00 to 19:15 in Main Conference Room.

LIABILITY AND INSURANCE

The Organizing Committee will accept no liability for injury or for loss or damage to property during the conference. Participants are advised to take out their own personal travel and health insurance.

MEMBERSHIP

Register and sign up to receive ISHEN Newsletter. Membership is free and open to everyone.
www.ishen.org

DEPARTURE

Bus for Aarhus Airport Tirstrup

Saturday June 2 at 8:30

For other departures, please contact ISHEN staff at the venue.

SOCIAL EVENTS

TUESDAY MAY 29 (ARRIVAL DAY)

Afternoon
Snacks, sandwiches and drinks are available.

Welcome Reception
All participants are cordially invited to the Welcome Reception in the Lobby from 18:30.

Dinner
Welcome and get-together dinner buffet from 20:00 in Restaurant A.

WEDNESDAY MAY 30

Breakfast from 6:30 in Restaurant A.

Lunch from 12:00-14:00 in Restaurant B.

Dinner: BBQ at 19:00 at the beach (weather permitting).

After Dinner Entertainment
Dance Lessons (*Sidsel Støy*).

THURSDAY MAY 31

Breakfast from 6:30 in Restaurant A.

Lunch from 12:00-14:00 in Restaurant B.

Dinner at 20:00 in Restaurant A.

After Dinner Entertainment
Classical singing (*Ladylike*) at 21:30 in the Lobby.

FRIDAY JUNE 1

Breakfast from 6:30 in Restaurant A.

Lunch from 12:00-14:00 in Restaurant B.

Dinner at 20:00 in Restaurant B – with Awards Ceremony.

Entertainment
Live band (*Trafikpop*) from 22:00 in Restaurant B.

SATURDAY JUNE 2 (DEPARTURE DAY)

Bus for Aarhus Airport Tirstrup **at 8:30**

NOTICE TO SPEAKERS AND POSTER PRESENTERS

ORAL PRESENTATION

Invited speakers
Your total presentation time is **20 minutes**.
The format is 15 minutes lecture and 5 minutes for discussion and comments.

Selected abstracts
Your total presentation time is **15 minutes**.
The format is 10 minutes lecture and 5 minutes for discussion and comments.

Note: You are required to include a **disclosure slide** indicating potential conflict of interests or the absence thereof at the beginning of your presentation.

Set-up
Upon arrival on site, we request that speakers check-in at the "Speaker Ready Room" for reviewing their presentation file with a USB Storage Device or CD and for uploading final version.

When reviewing your presentation, make sure that all materials (fonts, images and animations) are properly displayed on our equipment.

To avoid frequently occurring technical problems during presentations, speakers are requested to use the laptops present in the conference room for all presentations.

The files uploaded will not be used for any other purposes and will be deleted after the presentations.

Program files

The computers in the conference rooms are Microsoft Windows based with Microsoft Office 2010.

POSTER PRESENTATION

Poster dimensions: The poster panel space for your printed poster is 150 cm wide x 118 cm high (4' 11" wide and 3' 10" high). **Please ensure that your poster does not exceed the above size.**

Set-up
Posters will be displayed next to Restaurant B. Posters should be set-up upon arrival and remain on show for the duration of the meeting.

No audiovisual equipment will be available for poster presentations.

Presentation times:
All days 12:00-14:00 (during lunch).
Poster session 17:45-19:00 Thursday May 31.

PROGRAM AT A GLANCE

15th ISHEN Symposium

May 29 – June 2, 2012

Grenaa, Denmark

	Tuesday	Wednesday	Thursday	Friday	Saturday
6:30		Breakfast	Breakfast	Breakfast	Breakfast
7:00					
8:00			Session 5		Departure
9:00		Opening		Session 10	
		Session 1			
10:00	Arrival & Registration	Coffee	Coffee	Coffee	
		Session 2	Session 6	Session 11	
11:00					
12:00		Lunch & Poster Viewing	Lunch & Poster Viewing / Exec. C. Meeting	Lunch & Poster Viewing	
13:00					
14:00		Session 3	Session 7	Session 12	
15:00		Coffee	Coffee	Coffee	
16:00		Session 4	Session 8	Session 13	
17:00					
18:00	Welcome Reception		Poster Session (9)	Society Meeting	
19:00		Beach BBQ & After Dinner Entertainment			
20:00	Welcome & Get-Together Dinner Buffet		Dinner & After Dinner Entertainment	Dinner & After Dinner Entertainment	
21:00 - -					

SCIENTIFIC PROGRAM

May 29th Tuesday

Arrival and Registration

May 30th Wednesday

OPENING OF 15th ISHEN SYMPOSIUM

09:00-09:10 Welcome
Hendrik Vilstrup

09:10-09:20 Opening address from Aarhus University
Allan Flyvbjerg *(Dean, Health, Aarhus University)*

SESSION 1: HEPATIC ENCEPHALOPATHY – CONTEXTS
Chairs: Arne Schousboe / Hendrik Vilstrup

09:20-09:45 There and back again in hepatic encephalopathy (O1)
Roger F. Butterworth *(Montréal)*

09:45-10:10 Organic delirious states and other psychiatric disorders: lessons for the hepatologists (O2)
Raben Rosenberg *(Aarhus)*

10:10-10:30 **COFFEE BREAK**

SESSION 2: CEREBRAL METABOLISM AND HOMEOSTASIS OF AMMONIA
Chairs: Andreas Plaitakis / Arthur Cooper

10:30-10:50 Overview of cerebral ammonia metabolism (O3)
Peter Ott *(Aarhus)*

10:50-11:10 Human GDH isoforms and ammonia homeostasis (O4)
Ioannis Zaganas *(Heraklion)*

11:10-11:30 Glutaminase trafficking in brain supports novel roles in cerebral function (O5)
Javier Marquez *(Malaga)*

11:30-11:45 Evidence refuting the Trojan-Horse hypothesis of brain swelling in acute liver failure: K-type glutaminase is exclusively neuronal (O6)
Sharifi Y. et al. *(London)*

11:45-12:00 Brain osmolytes and brain edema in a rat model of chronic liver failure: in vivo longitudinal 1H spectroscopic imaging and diffusion tensor imaging studies at 9.4T (O7)
Cudalbu C. et al. *(Lausanne)*

12:00-14:00 **LUNCH AND POSTER VIEWING**

SESSION 3: MECHANISMS OF AMMONIA TOXICITY IN THE BRAIN

Chairs: Jan Albrecht / Michael D. Norenberg

14:00-14:20 Ammonia and glutamatergic synaptic transmission (O8)
Vicente Felipo *(Valencia)*

14:20-14:40 Role of endothelial cells in the astrocyte swelling and brain edema associated with acute liver failure (O9)
Arumugam. R. Jayakumar *(Miami)*

14:40-15:00 Microglia in the mechanism of astrocyte swelling/brain edema in acute liver failure (O10)
K. V. Rama Rao *(Miami)*

15:00-15:15 Glutamate-induced, NMDA receptor-mediated decrease of KIR4.1 channel expression in astrocytes: does it reflect the sequence of events in hepatic encephalopathy? (O11)
Obara-Michlewska M. et al. *(Warsaw)*

15:15-15:30 Selective decrease of glutathione content in prefrontal cortical mitochondria of HE rats: prevention by histidine (O12)
Ruszkiewicz J. et al. *(Warsaw)*

15:30-16:00 **COFFEE BREAK**

SESSION 4: BRAIN METABOLISM: LESSONS FROM HUMAN STUDIES

Chairs: Susanne Keiding / Juan Cordoba

16:00-16:20 Urea-cycle defects and hyperammonemia: lessons for the hepatologist (O13)
Marshall Summar *(Washington DC)*

16:20-16:40 PET studies of cirrhotic patients with and without HE (O14)
Michael Sørensen *(Aarhus)*

16:40-17:00 Lessons from nitrogen challenges (O15)
Christopher Record *(Newcastle)*

17:00-17:15 Metaanalysis of magnetic resonance imaging and neurological outcomes in liver failure and severe hyperammonemia (O16)
Kandiah P.A. et al. *(Georgia)*

17:15-17:30 Brain magnetic resonance in episodic hepatic encephalopathy (O17)
Chavarria L. et al. *(Barcelona)*

May 31st Thursday

SESSION 5: HYPERAMMONEMIA: RATIONALE FOR TREATMENT

Chairs: Barjesh C. Sharma / Stephen Olde-Damink

08:30-08:50 Systematic review of randomised controlled trials on oral branched-chain amino acids for recurrent hepatic encephalopathy (O18)
Lise Lotte Gluud *(Copenhagen)*

08:50-09:10 Ornithine-phenyl-acetate revisited (O19)
Rajiv Jalan *(London)*

09:10-09:30 L-acetylcarnitine in hepatic encephalopathy (O20)
Michele Malaguanera *(Catania)*

09:30-09:45 Protein malnutrition increases the risk of overt and minimal hepatic encephalopathy (O21)
Lucidi C. et al. *(Rome)*

09:45-10:00 Metformin protects against hepatic encephalopathy: impact on insulin resistance and glutaminase activity (O22)
Ampuero J. et al. *(Seville)*

10:00-10:30 **COFFEE BREAK**

SESSION 6: AMMONIA AND CEREBRAL ENERGY METABOLISM

Chairs: Roger F. Butterworth / Helle Waagepetersen

10:30-10:50 Effects of ammonia on energy metabolism and synthesis of neurotransmitter GABA in mouse cerebral cortical cell cultures (O23)
Renata Leke *(Porto Alegre)*

10:50-11:10 Ammonia and oxidative stress (O24)
Christopher Rose *(Montréal)*

11:10-11:25 HE is associated with decreased brain oxygen metabolism and blood flow, not increased ammonia uptake (O25)
Dam G. et al. *(Aarhus)*

11:25-11:40 Increased cerebral lactate contributes to brain edema in cirrhotic rats (O26)
Bosoi C.R. et al. *(Montréal)*

12:00-14:00 **LUNCH AND POSTER VIEWEING**

EXECUTIVE COMMITTEE MEETING WITH LUNCH

SESSION 7: CEREBRAL AMMONIA CONVERSION

Chairs: Arne Schousboe / Vicente Felipo

14:00-14:20 Possible treatment of end-stage hyperammonemic encephalopathy by inhibition of glutamine synthetase (O27)
Arthur Cooper *(New York)*

14:20-14:40 Alanine as an alternative ammonia scavenger (O28)
Lasse K. Bak *(Copenhagen)*

14:40-14:55 Does inhibition of glutamine synthesis induce glutamate dehydrogenase-dependent ammonia fixation into alanine in an in vivo rat model of hyperammonemia? (O29)
Fries A. et al. *(Aarhus)*

14:55-15:10 The effect of ammonium on extracellular lactate, adenosine and glutamate in cortex of rat brain slices. A biosensor dose-response study (O30)
Bjerring PN et al. *(Copenhagen)*

15:10-15:40 **COFFEE BREAK**

SESSION 8: MINIMAL HEPATIC ENCEPHALOPATHY

Chairs: Simon Taylor-Robinson / Michael Sørensen

15:40-16:00 Psychometric tests for Mhe (O31)
Karin Weissenborn *(Hannover)*

16:00-16:20 Cognitive impairments and HE (O32)
Oliviero Riggio *(Rome)*

16:20-16:40 Circadian rythms in patients with cirrhosis (O33)
Sara Montagnese *(Padua)*

16:40-16:55 Correlation between degree and quality of sleep disturbance and the level of neuropsychiatric impairment in patients of cirrhosis (O34)
Samanta J. et al. *(Chandigarh)*

16:55-17:10 Flicker fusion frequencies for the diagnosis of hepatic encephalopathy: confounding variables and best performance measures (O35)
Halliday E. J. et al. *(London)*

17:10-17:25 The utility of evoked potential for the diagnosis of hepatic encephalopathy – it's all in the processing (O36)
Rayan M. et al. *(Westminster)*

SESSION 9: POSTER SESSION

17:45-19:00 **Poster Session / with wine & cheese**

June 1st Friday

SESSION 10: GUT-LIVER-AXIS: RATIONALE FOR TREATMENT

Chairs: Kevin Mullen / Marsha Y. Morgan

09:00-09:20 Importance of gut flora in hepatic encephalopathy – new results (O37)
Radha K. Dhiman *(Chandigarh)*

09:20-09:40 Role of antibiotics (O38)
Jasmohan S. Bajaj *(Richmond)*

09:40-10:00 Nonabsorbable disaccharides and hepatic encephalopathy (O39)
Praveen Sharma *(New Delhi)*

10:00-10:15 Rifaximin for the treatment of hepatic encephalopathy; a meta-analysis of randomized studies (O40)
Morgan M. et al. *(London)*

10:15-10:30 Toll-like receptor-4: a novel target for therapy of hepatic encephalopathy (O41)
Sharifi Y. et al. *(London)*

10:30-10:50 **COFFEE BREAK**

SESSION 11: ELECTROLYTES, WATER CONTENT AND TRANSPORTERS

Chairs: Peter Ott / Christopher Rose

10:50-11:10 Urea-cycle defects and hyperammonemia: effects on functional imaging (O42)
Andrea Gropman *(Washington DC)*

11:10-11:30 Oxidative metabolism and brain oedema (O43)
Peter Bjerring *(Copenhagen)*

11:30-11:45 Hyponatraemia, hepatitis C, and diabetes are risk factors for hepatic encephalopathy in large prospective studies in decompensated cirrhosis (O44)
Watson H. et al.

11:45-12:00 Increased expression of aquaporin-4 in perivascular astrocytes end-feet contribute to the development of brain edema in acute liver failure (O45)
Thumburu K. K. et al. *(Chandigarh)*

12:00-14:00 **LUNCH AND POSTER SESSION**

SESSION 12: THE BRAIN IN LIVER FAILURE

Chairs: Fin S. Larsen / Radha K. Dhiman

14:00-14:20 Liver failure and the brain: a look through the crystal ball (O46)
Debbie L. Shawcross *(London)*

14:20-14:40 Does liver assist help patients with HE? (O47)
Fin Stolze Larsen *(Copenhagen)*

14:40-15:00 Neuroinflammation in the pathogenesis of the CNS complications of acute liver failure (O48)
Roger F. Butterworth *(Montréal)*

15:00-15:15 Hepatic encephalopathy recedes after liver transplantation but cognitive function does not normalize (O49)
Tryc A. B. et al. *(Hannover)*

15:15-15:30 Role of oxidative stress in prevention of brain edema during an acute deterioration of chronic liver failure (O50)
Bosoi C. R. et al. *(Montréal)*

15:30-16:00 **COFFEE BREAK**

SESSION 13: INFLAMMATION AND HEPATIC ENCEPHALOPATHY

Chairs: Rajiv Jalan / Roger F. Butterworth

16:00-16:20 Neuroinflammation in cognitive impairment and hypokinesia in hyperammonemia and hepatic encephalopathy (O51)
Vicente Felipo *(Valencia)*

16:20-16:40 Systemic inflammation and ammonia (O52)
Debbie L. Shawcross *(London)*

16:40-17:00 Encephalopathy and liver transplant (O53)
Juan Cordoba *(Barcelona)*

17:00-17:15 Bacterial infections increase the incidence of both overt and minimal hepatic encephalopathy: results of a prospective study (O54)
Lucidi C. et al. *(Rome)*

17:15-17:30 Systemic oxidative stress induction leads to brain edema in hyperammonemic portacaval-shunted rats (O55)
Bosoi C.R. et al. *(Montréal)*

18:00-19:15 SOCIETY MEETING

ABSTRACTS – ORAL PRESENTATIONS

SESSION 1: HEPATIC ENCEPHALOPATHY - CONTEXTS

O1 THERE AND BACK AGAIN IN HEPATIC ENCEPHALOPATHY

Roger F. Butterworth
Universite de Montreal

Ground-breaking discoveries in the 70's and 80's based upon careful clinical assessments and simple neurophysiologic testing procedures by dedicated *Hobbits from both sides of the Atlantic remain the basis of our current understanding of the mechanisms underlying the causes of encephalopathy in liver failure. For example, the effectiveness of L-DOPA in improving cognitive and motor function in patients with liver failure first described in 1973 led to the identification of a dopaminergic lesion as a key component in the pathogenesis of hepatic encephalopathy, a notion that was subsequently confirmed using both classical biochemical and neuroimaging techniques. These findings led to coining of the term "cirrhosis-related parkinsonism". However, the prevalence of the disorder, the identification of patients likely to benefit from L-DOPA therapy and the precise relationship between the failing liver, dopaminergic neuronal cell death and the parkinsonism resulting from decompensated cirrhosis remains a mystery. Some years later, similarities between visual-evoked potentials in rabbits with toxic liver injury to evoked potentials from the same rabbits treated with GABA receptor agonists led investigators in 1983 to formulate the notion of "increased GABAergic tone" as a second major cause of encephalopathy in liver failure. This phenomenon now appears to result from accumulation in the brain of endogenous substances with GABA receptor-modulating properties. However, the identity of these substances, the relationship between their accumulation in the brain and other consequences of the failing liver as well as the development of novel agents with the requisite GABA-lowering properties as novel therapeutics in liver failure continue to elude us. Adventures by future generations of *Hobbits from around the world will undoubtedly solve these mysteries and return with new treatments for the CNS complications of liver failure.
**With apologies to J.R.R. Tolkien.*

O2 ORGANIC DELIRIOUS STATES AND OTHER PSYCHIATRIC DISORDERS: LESSONS FOR THE HEPATOLOGISTS

Raben Rosenberg
Centre for Psychiatric Research, Aarhus University Hospital Risskov, DK-8240 Risskov, Denmark

Hepatic encephalopathy (HE) is a serious complication of liver disease with a variety of neuropsychiatric symptoms as indicated by its name. From a psychiatric perspective its nosological status calls for clarification. Is HE just another example of a delirium due to the underlying somatic illness or a specific nosological entity that can be clearly differentiated from psychiatric disorders such as other delirious states, organic cognitive and emotional disorders including dementia, depression or personality change due to liver disease? Will a reconceptualization of HE in terms of current psychiatric nosology have any implication for pathophysiological understanding and treatment?

SESSION 2: CEREBRAL METABOLISM AND HOMEOSTATSIS OF AMMONIA

O3 OVERVIEW OF CEREBRAL AMMONIA METABOLISM

Peter Ott
Department of Medicine V (Hepatology), Aarhus University Hospital, Aarhus, Denmark

The cerebral influx of ammonia is primarily driven by is arterial concentration and CBF. Transmembrane transport probably includes both diffusion of NH3 and – like in the kidney and the liver - transport of NH4+ by specific membrane proteins. Intracerebral pH, membrane potential and cerebral ammonia production contribute to a brain:blood ratio of ammonia above 1. Astrocytic detoxification of ammonia by amidation of glutamate increases cerebral glutamine concentrations dramatically. Several hypotheses relate to elevated glutamine: The osmotic effect could contribute to astrocytic swelling. Glutamine translocation into astrocytic mitochondria could have deleterious effects ("Trojan Horse"). Also, important interactions with neurons through the glutamate- glutamine and GABA- glutamate cycles could be disturbed, leading to increased activity of both excitatory glutamate and inhibitory GABA. Inhibition of glutamine synthesis abolish some of these effects.
Ammonia affects energy metabolism. Lactate is paradoxically elevated, even though hypoxia can be excluded. Lactate correlate to glutamine and intracranial pressure. Formation of glutamine removes carbon skeletons from the TCA cycle. According to one hypothesis, the TCA cycle is inhibited to such a degree that conversion of pyruvate to lactate is necessary to maintain glycolytic ATP production. This is supported by experimental reports that ammonia inhibits α-ketogluterate dehydrogenase and pyruvate dehydrogenase. Also, in patients with ALF, the cerebral efflux of glutamine was several fold higher than the influx of ammonia, suggesting oxidative metabolism of amino acids. However, recent studies employing stable isotopes in fact demonstrated enhanced TCA cycle activity. Ammonia accelerates glycolysis and this could lead to elevated lactate and pyruvate. Since CMR for glucose and oxygen is reduced in the clinical setting of HE and ALF, our understanding of this issue is incomplete.
Two-hit hypotheses propose that ammonia toxicity is enhanced by other factors. The candidates are inflammation (both systemic and neuroinflammation), oxidative stress (both systemic and microglial) and hyponatraemia.

O4 HUMAN GLUTAMATE DEHYDROGENASE ISOFORMS AND AMMONIA HOMEOSTASIS

Ioannis Zaganas and Andreas Plaitakis
Neurology Laboratory, Medical School, University of Crete, Heraklion, Crete, Greece

Glutamate dehydrogenase (GDH), a highly abundant mitochondrial enzyme, catalyses the reversible reductive amination of α-ketoglutarate to glutamate and is thought to play a key role in cellular ammonia metabolism. In addition to the house keeping GLUD1 gene, humans and great apes, in contrast to other mammals, possess a second intronless GLUD2 gene. Despite their high amino acid homology, the isoenzymes encoded by the GLUD1 and GLUD2 genes (hGDH1 and hGDH2, respectively) display great differences in their enzymatic properties. Furthermore, they show a differential tissue distribution, with the hGDH2 protein shown recently to be present in astrocytes in brain, in Sertoli cells in testis and in epithelial cells in kidney. Both human isoenzymes display a high Km for ammonia, an observation suggesting that the direction of the catalyzed reaction in vivo is towards oxidative deamination of glutamate. However, it is possible that hyperammonemic states or even local increases in ammonia concentration, as for example in the context of a multi-molecular complex (metabolon), could permit the hGDH-catalyzed fixation of ammonia to form glutamate. In addition, the

unique enzymatic properties of hGDH2 (resistance to GTP, dependence on ADP for function and better functioning in lower pH compared to hGDH1) could permit its adaptation to novel human-specific metabolic requirements. For example, hGDH2 could contribute to regulation of ammonia homeostasis in the kidney, by enabling the production of ammonia under conditions (relative acidification and/or high GTP levels) that inhibit hGDH1. By analogy, in patients with the hyperinsulinism/hypermmonemia syndrome harbouring GTP-resistant mutants of hGDH1, the source of hyperammonemia is thought to be the kidney. Finally, the unique properties of hGDH2 could permit its function under the unique conditions prevailing in glutamatergic synapses during neurotransmission.

O5 GLUTAMINASE TRAFFICKING IN BRAIN SUPPORTS NOVEL ROLES IN CEREBRAL FUNCTION

Javier Márquez, Carolina Cardona, Mercedes Martín-Rufián, Carolina Lobo, Antonia Gutiérrez, José A. Campos-Sandoval
Departamento de Biología Molecular y Bioquímica, Laboratorio de Química de Proteínas, Facultad de Ciencias, Universidad de Málaga, 29071 Málaga, Spain

Glutamine/glutamate homeostasis must be exquisitely regulated in mammalian brain and glutaminase (GA, E.C. 3.5.1.2) is one of the main enzymes involved. GA is considered as the main glutamate-producer enzyme in brain and, consequently, it is essential for both glutamatergic and gabaergic transmissions. The classical pattern of GA expression in mammals has been recently challenged by the discovery of novel transcript variants and isoforms with particular relevance in brain. Furthermore, the interactome of brain GA is also starting to be uncovered adding a new level of regulatory complexity. GA may traffic in brain and unexpected locations for GA, like cytosol and nucleus, have been found for GA isoforms. Protein-interacting partners for distinct GA isoforms have been identified and implicated in their selective targeting and novel roles performed by GA outside the mitochondria. All these experimental evidences suggest new functions for brain GA, mainly in transcriptional control, vascular regulation and neuronal development. In this talk, we summarize recent findings that point consistently towards GA as a multifaceted protein able to perform different tasks.
Supported by Grants: SAF 2010-17573 (Spanish Ministry of Science and Innovation), CVI-6656 (Regional Government of Andalusia) and RD06/0001/1012 (Spanish Health Institute Carlos III).

O6 EVIDENCE REFUTING THE TROJAN-HORSE HYPOTHESIS OF BRAIN SWELLING IN ACUTE LIVER FAILURE: K-TYPE GLUTAMINASE IS EXCLUSIVELY NEURONAL

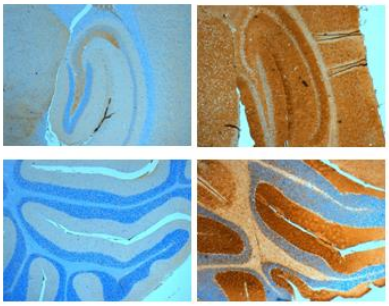
Y Sharifi, N Shah, M Jover, D Marsden, N Davies, F Scaravilli & R Jalan

Introduction: Astrocytic swelling is the characteristic feature of hyperammonemia and acute liver failure (ALF) which is thought to result from accumulation of glutamine due to the action of astrocytic glutamine synthetase (GS). It has been suggested that glutamine may not be a benign amino acid and may act as “Trojan horse” which leads to astrocytic apoptosis as it is metabolised by Glutaminase (GLN) yielding glutamate and ammonia. In vivo proof for this hypothesis is lacking. In health, GLN is mainly neuronal and generates glutamate and GABA. The aims of the study were to define the expression of the ammonia metabolising enzymes, GS and GLN in the brain of ALF animals.
Material and methods: Two groups of CD11 mice were studied, sham: n=6; paracetamol (500 mg/kg IP): n=7. The animals were maintained normothermic and resuscitated with fluid and glucose and, sacrificed at 8 hour after injection of APAP. Arterial ammonia (COBAS) and frontal cortex brain water (dry weight technique) were measured. The brain sections

were stained for GS and GLN.
Results: Arterial ammonia was significantly higher in the ALF group compared with controls (345±32 vs. 132± 11 p=0.002) and brain water (83.6 ± 2.3 vs. 76.3 ± 2.6 p=0.05). GS protein expression was observed in the astrocytes globally in both groups and was not different but was also seen in the oligodendrocytes only in ALF group. K-type GLN was expressed only in the neurons and not in the astrocytes and was significantly higher in the ALF animals (++++) compared with controls (+). The most marked areas were the hippocampus and thalamus interestingly the staining was mainly axonal.

K-glutaminase : sham vs APAP brain

Sham *APAP*



Conclusion: The results of this study refute the Trojan-horse hypothesis and show for the first time increased protein expression of K-type GLN which is exclusively neuronal. From the pathophysiological perspective, this may function to generate excessive ammonia in the neuron thereby producing neuronal cell death.

O7 BRAIN OSMOLYTES AND BRAIN EDEMA IN A RAT MODEL OF CHRONIC LIVER FAILURE: IN VIVO LONGITUDINAL 1H SPECTROSCOPIC IMAGING AND DIFFUSION TENSOR IMAGING STUDIES AT 9.4T

Cristina Cudalbu[1], Olivier Braissant[2], Mario Lepore[1], Rolf Gruetter[1,3] and Valérie A. McLin[4]

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Introduction: Chronic liver disease (CLD) in children and adults is characterized by an array of cognitive and fine motor deficits labeled as hepatic encephalopathy (HE) [1,2]. Increasing evidence points to ammonia as a culprit in HE [3]. Astrocytes detoxify ammonia by generating glutamine. Human studies have inconsistently shown cerebral glutamine increase and osmolyte reduction [4,5]. Thus, the aim of this study was to analyze the in vivo alterations in brain osmolytes and brain edema in a rat model of CLD using longitudinal 1H Spectroscopic Imaging (SI) and Diffusion tensor Imaging (DTI).
Methods: Wistar rats were bile duct ligated (BDL) and scanned before and weekly after BDL (up to 8weeks). In vivo 1H SI was performed (TE=2.8ms)[6] at 9.4T resulting in metabolic maps from 5×10 voxels (voxel size 0.75×0.75×2mm3=1.1µL). Metabolite concentrations were calculated using LCModel. DTI was performed with double-spin-echo-semi-adiabatic 4 shots EPI sequence [7] and diffusivity values (ADC-apparent diffusion coefficient, FA-fractional anisotropy) were derived from the tensor. ADC was measured in cortex, striatum and hippocampus.

Results and Discussion: In vivo spatial distribution of 12 metabolites (i.e. Gln, Glu, Cr, PCr, tCho, Ins, Tau, Lac, NAA+NAAG, PE, Glc, GABA) was obtained in the somatosensory and retrosplenial cortex, hippocampus and thalamus before and after BDL. The maps for Gln showed an increased at all-time points after BDL reaching more than 300% at 8 weeks. Ins, tCho and Tau decreased significantly over time. The sum of the main brain osmolytes (Gln, Ins, tCho, Tau) was constant over time, presumably to compensate for the Gln increase. The maps of other brain metabolites did not show any visible difference between and after BDL. ADC values showed a slight increase over the first 8 weeks after BDL, suggesting that mild edema is noticeable in spite of ongoing osmotic regulation.
Conclusion: We conclude that prior to the appearance of severe neurological signs in CLD, the osmotic imbalance created by the continuous increase of Gln is likely to be compensated by a concomitant decrease of other idiogenic osmolytes resulting in minimal brain edema.

[1]Norenberg MD et al, Metab Brain Dis 2009; [2]Caudle SE et al, J Pediatr. 2010; [3]Braissant O, Mol Genet Metab. 2010; [4]Rovira A et al, AJNR 29, 2008; [5]Zwingmann C, J Neurosci Res. 2007; [6]Mlynárik V et al. Magn Reson Med. 59:52, 2008; [7]van de Looij Y et al, Magn Reson Med, 2011.
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SESSION 3: MECHANISMS OF AMMONIA TOXICITY IN THE BRAIN

O8 AMMONIA AND GLUTAMATERGIC NEUROTRANSMISSION

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Hyperammonemia affects glutamatergic neurotransmission at many steps, including extracellular glutamate concentration, transport and transporters of glutamate, content and function of different types of glutamate receptors and function of signal transduction pathways associated to glutamate receptors. Alterations in glutamatergic neurotransmission play a main role in the neurological alterations in chronic hyperammonemia and hepatic encephalopathy (HE) and in the mechanisms leading to death in acute hyperammonemia or liver failure. The alterations in glutamatergic neurotransmission induced by acute and chronic hyperammonemia are different and, in some cases, are even opposite. Acute hyperammonemia and liver failure lead to excessive activation of NMDA receptors in brain, which is responsible for death in acute ammonia intoxication and contributes to death in acute liver failure (ALF). Blocking NMDA receptors prevents death in acute hyperammonemia and in mild ALF and delays death in strong ALF. Chronic moderate hyperammonemia and hepatic encephalopathy affects most types of glutamate receptors: NMDA, AMPA and metabotropic glutamate receptors (mGluRs) in different ways. Alterations in glutamatergic neurotransmission involving mGluRs are responsible for hypolocomotion in rats with chronic hyperammonemia or HE. Extracellular glutamate is increased in substantia nigra pars reticulata (SNr) of rats with HE, leading to excessive activation of metabotropic receptor 1 (mGluR1). Blocking this receptor in SNr normalizes motor activity. Alterations in glutamatergic neurotransmission involving NMDA receptors are responsible for some types of cognitive impairment by at least two different mechanisms: impairment of long-term potentiation in hippocampus and of the glutamate-NO-cGMP pathway in cerebellum. Chronic hyperammonemia alters the phosphorylation, membrane expression and function of NMDA receptors, resulting in enhanced tonic activation of NMDA receptors in cerebellum in vivo and reduced function of the glutamate-NO-cGMP pathway which is responsible for impaired ability to learn a Y maze conditional discrimination task in chronic hyperammonemia and HE. Treatments that restore this pathway also restore learning ability.

O9 ROLE OF ENDOTHELIAL CELLS IN THE ASTROCYTE SWELLING AND BRAIN EDEMA ASSOCIATED WITH ACUTE LIVER FAILURE

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Brain edema is an important complication of acute hepatic encephalopathy (acute liver failure, ALF), and astrocyte swelling is largely responsible for its development. Elevated blood and brain ammonia levels have been considered as major etiological factors in this edema. In addition to ammonia, recent studies have suggested that systemic infection, inflammation, as well as endotoxin (lipopolysaccharide, LPS) are also involved in ALF-associated brain edema. As endothelial cells (ECs) are the first resident brain cells exposed to blood-borne “noxious agents” (i.e., ammonia, cytokines, LPS) that are present in ALF, these cells may be in a critical position to react to these agents and

trigger a process resulting in astrocyte swelling/brain edema. We therefore examined the effect of conditioned media (CM) from ammonia, LPS and cytokine-treated cultured endothelial cells on cell swelling in cultured astrocytes. CM from ammonia-treated ECs when added to astrocytes caused significant cell swelling, and such swelling was potentiated when astrocytes were exposed to CM from ECs-treated with a combination of ammonia, LPS and CKs. We also found an additive effect when astrocytes were exposed to ammonia along with CM from ammonia-treated ECs. Additionally, ECs treated with ammonia showed a significant increase in the production of oxy-radicals, nitric oxide, as well as evidence of oxidative/nitrative stress and activation of the transcription factor NF-kappaB. CM derived from ECs treated with ammonia, along with antioxidants or the NF-kappaB inhibitor BAY 11-7082, when added to astrocytes resulted in a significant reduction in cell swelling, as compared to the effect of CM from ECs-treated only with ammonia. We also identified increased nuclear NF-kappaB expression (activation) in rat brain cortical ECs in the thioacetamide model of ALF. These studies suggest that endothelial cells significantly contribute to the astrocyte swelling/brain edema in ALF, likely as a consequence of oxidative/nitrative stress and activation of NF-kappaB.

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O10 MICROGLIA IN THE MECHANISM OF ASTROCYTE SWELLING/BRAIN EDEMA IN ACUTE LIVER FAILURE

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Brain edema is a lethal complication of the acute form of hepatic encephalopathy, acute liver failure (ALF). The edema in ALF is believed to be largely cytotoxic due to the swelling of astrocytes. Ammonia, a neurotoxin whose levels are elevated in blood and brains of patients with ALF, has been strongly implicated in the development of the brain edema. It was previously shown that a pathophysiological level of ammonia causes cell swelling in cultured astrocytes. While mechanisms of astrocyte swelling/brain edema in ALF are not completely clear, emerging evidence suggests that inflammation resulting from necrotic liver and/or systemic sepsis contributes to the edema. Since microglia represent the major neural cell type capable of evoking an inflammatory response, and since recent reports document the activation of microglia in experimental models of ALF and hyperammonemia, we investigated potential role of microglia in the mechanism of ammonia-induced astrocyte swelling. Primary microglial cultures were treated with 5 mM NH4Cl, and conditioned media (CM) from control and ammonia-treated microglia were added to primary cultures of astrocytes and cell volume determined. Addition of CM from ammonia-treated microglia to normal astrocytes caused a significant swelling (34 and 41% $p<0.05$) at 12 and 24 h, respectively, while the CM from microglia treated with ammonia for 3 and 6 did not result in astrocyte swelling. Astrocyte swelling was synergistically increased (77%, $p<0.01$) when astrocytes were additionally treated with 5 mM ammonia. CM from ammonia-treated microglia also showed a significant increase in the synthesis and release of oxy-radicals and nitric oxide in the CM. Further, addition of CM from ammonia-treated microglia containing Tempol (a superoxide scavenger) or uric acid (a peroxynitrite scavenger) to astrocytes resulted in a marked reduction in cell swelling (77-80%, $p<0.001$). Together, these studies indicate that microglia contribute to the ammonia-induced astrocyte swelling by a mechanism involving oxidative/nitrosative stress. Microglia may represent a therapeutic target for the astrocyte swelling/brain edema associated with ALF.

O11 GLUTAMATE-INDUCED, NMDA RECEPTOR-MEDIATED DECREASE OF KIR4.1 CHANNEL EXPRESSION IN ASTROCYTES: DOES IT REFLECT THE SEQUENCE OF EVENTS IN HEPATIC ENCEPHALOPATHY ?

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The inwardly rectifying potassium channel Kir4.1 is implicated in the maintenance of ion homeostasis and volume control of astrocytes (Obara-Michlewska M. et al., J. Neurosci. Res., 2011). Decreased expression of Kir4.1 was noted in the thioacetamide (TAA) model of hepatic encephalopathy (HE), and in cultured astrocytes treated with glutamine (Gln), but not with ammonia alone. We speculated that excess glutamate (Glu), which extracellular concentration goes up in TAA-induced HE (Hilgier W. et al., J. Neurosci Res., 1999) could contribute to Kir4.1 depression in both experimental settings. Here we show that treatment of cultured astrocytes with Glu or NMDA reduces Kir4.1 expression, and the effect is attenuated by antagonists of the NMDA receptor (MK-801 and AP-5) but not by a mGluR group I antagonist (MTEP), and by a non-transportable (TBOA) but not by a transportable Glu uptake inhibitor (PDC). Neither basal Kir4.1 expression nor the effect of Glu on its expression were affected by histone deacetylase inhibitors (trichostatin A, valproate, phenylbutyrate), which are protective in microglia and neurons. The antioxidants taurine and apocynin, and the nitric oxide synthase inhibitor (L-NNA) did not modulate the effect of Glu. However, Kir4.1 expression was decreased, and the effect of Glu on Kir4.1 expression was potentiated, by glutathione diethyl ester, an antioxidant which, like Gln, is degraded intracellularly to Glu. Preliminary results revealed a tendency towards reversal of the decrease of Kir4.1 expression in the cerebral cortex of TAA-treated rats upon intraperitoneal co-administration of the NMDA receptor antagonist, memantine. The results support the notion that excessive Glu accumulating in HE may decrease astrocytic Kir4.1 expression by a mechanism encompassing NMDA receptor signaling, aided by an unidentified precedent intracellular action of Glu.

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O12 SELECTIVE DECREASE OF GLUTATHIONE CONTENT IN PREFRONTAL CORTICAL MITOCHONDRIA OF HE RATS: PREVENTION BY HISTIDINE

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Increasing evidence suggests that the pool of glutathione residing in mitochondria (mGSH) plays a key role in cell defense against oxidative and nitrosative stress (ONS). ONS is a key pathogenic event in hepatic encephalopathy (HE), as convincingly demonstrated in previous studies in the thioacetamide (TAA) model (Rama Rao et al., Am J. Pathol., 2010, 176(3):1400-1408, and references therein). We measured GSH content in the homogenates and in Percoll gradient-purified mitochondrial fractions derived from prefrontal cortex (pfc) and striatum (str) of rats with TAA-induced HE (3 i.p. injections of 250 mg/kg b.w. at 24h intervals, HE rats; Hilgier and Olson, J. Neurochem.,1994, 62:197-204). Mitochondria derived from pfc, but not from str of HE rats showed a 54% decrease of mGSH as compared to control rats, whereas the homogenate GSH was not affected in either structure. The vulnerability of mGSH in pfc coincided with brain tissue edema which in this model specifically affects the cerebral cortex (Hilgier and Olson, J. Neurochem.,1994, 62:197-204). Treatment with histidine (His) attenuated the decrease of mGSH, consistent with the previously demonstrated

ability of the compound to ameliorate HE symptoms in this model (Rama Rao et al., Am J. Pathol., 2010, 176(3):1400-1408). His did not increase GSH content in the pfc and str homogenates of HE rats. The results indicate that adequate GSH supply to mitochondria plays an important role in protecting the brain against ONS associated with HE. Preliminary data indicate that the HE-induced decrease of mGSH is not associated with altered expression of mRNAs coding proteins transporting GSH from cytoplasm to mitochondria.

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SESSION 4: BRAIN METABOLISM: LESSONS FROM HUMAN STUDIES

O13 WHAT UREA CYCLE DISORDERS TEACH US ABOUT HEPATIC ENCEPHALOPATHY

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UCD's are a rare but widely distributed group of disorders affecting the metabolism of ammonia and the production of urea cycle intermediates. In the field of biochemical genetics we have been caring for these patients for many decades and have developed an NIH sponsored longitudinal study to track their natural history and outcomes. This talk will present data on the consequences of isolated UCD metabolism and how this overlaps with the wider field of hepatic encephalopathy. The outcome of acute hyper-ammonemia results in long term damage to the central nervous system in both children and adults. The more insidious effects of chronic hyperammonemia in UCD patients and in X-linked OTC carriers previously thought to be normal will be described. The effects of UCD disruption on nitric oxide metabolism and its secondary effects will be described. Finally the basis and results of UCD specific treatments will be outlined and how they might potentially impact HE.

O14 PET STUDIES OF CIRRHOTIC PATIENTS WITH AND WITHOUT HE

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Positron emission tomography (PET) is an excellent tool for noninvasive, real-time *in vivo* measurements of tissue blood perfusion and metabolic processes.
In this paper, the use of PET for studies of cerebral blood flow, glucose metabolism, oxygen consumption and ammonia metabolism in studies of hepatic encephalopathy (HE) pathogenesis is reviewed. In particular, the potential changes in energy metabolism are addressed.
Cerebral blood flow and oxygen metabolism have both been shown to be decreased in HE when compared to healthy subjects and cirrhosis patients without HE. Interestingly, the values return to values similar to the two control groups, when patients recover from HE, indicating alterations in cerebral oxidative metabolism during HE.
Fluorodeoxyglucose (^{18}F-FDG) PET studies of cerebral glucose metabolism have shown decreased metabolic rate of glucose in certain brain areas in patients with early stages of HE in agreement with the cerebral symptoms observed. Recent PET studies agree that the blood-brain barrier for ammonia is unchanged in patients with cirrhosis with and without HE when compared to healthy subjects, and the studies have not been able to show a direct link between cerebral ammonia metabolism and HE, but the interpretation of the results is complex because many parameters may be altered. Whereas most PET studies show potential correlations between different metabolic parameters, the pathogenetic importance is difficult to decide without interventions.
The number of PET studies of hepatic encephalopathy (HE) is rather limited, probably because the technique is not commonly available, it is considered expensive and it may be difficult to get patients with HE to volunteer for such relatively long examinations. However, the power of the technique for clinical studies of HE is very strong and more studies are thus encouraged.

O15 LESSONS FROM NITROGEN CHALLENGES

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For many years the importance of ammonia in hepatic encephalopathy has been controversial and at the 9th Intrnational Symposium on Ammonia in 1996 in Newcastle, Professor Sheila Sherlock advised deletion of ammonia from the Society's name. The effect of induction of hyperammonaemia on brain function and metabolism in patients with stable cirrhosis has been explored in a series of experiments.
Blood ammonia is derived from:
1. Conversion of ingested glutamine to glutamate in small intestine (Oppong et al Hepatology 1997; 26: 870-76; Plauth et al Gut 2000; 46: 849-55)
2. Up regulation of intestinal glutaminase (Romero Gomez et al J Hepatol 2004; 41: 49-54)
3. Catabolism of dietary amino acids (Mardini et al Met Brain Disease 2006; 21: 1-10)
4. Bacterial ureolysis in the colon (Walser and Badenlos J Clin Invest 1958; 38:1617-26

After glutamine challenge blood ammonia doubled and this was associated with a prolongation in reaction time which was ameliorated by liver transplantation and administration of L ornithine L aspartate (Gut 2000;47:571–574). In these experiments the effect upon brain function could have been mediated by glutamine rather than ammonia but after administration of a glutamine free amino acid mixture resembling haemoglobin (a known precipitant of HE), hyperammonaemia resulted in prolonged reaction times and EEG slowing (J Hepatol 2001;34:658-64). In order to maximize hyperammonaemia we administerd a mixture of serine, threonine and glycine and have shown an increase in brain water as shown by diffusion tensor imaging which was accompanied by a rapid fall in brain myoinositol and both brain diffusivity and myoinositol correlated with blood ammonia concentration. The change in blood ammonia correlated with the change in brain glutamine (J Hepatol 2011; 54:1154–1160). These results support the importance of ammonia in the pathogenesis of hepatic encephalopathy.

O16 METAANALYSIS OF MAGNETIC RESONANCE IMAGING FINDINGS AND NEUROLOGICAL OUTCOMES IN LIVER FAILURE AND SEVERE HYPERAMMONEMIA

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Back ground: The neurotoxic effect of ammonia with resultant cytotoxic edema has been reproducibly demonstrated in animal models. Conversely, MRI findings and neurological sequelae in hyperammonemia due to liver failure remains poorly characterized adding to the clinical misgivings about the utility of plasma ammonia levels. By means of a meta-analysis, we attempt to better delineate a correlation between a distinctive pattern of brain injury, hyperammonemia and neurological outcome.
Methods: In addition to clinical data obtained from 9 patients collected retrospectively, a thorough keyword PUBMED search of all publications in English between Jan 1990-Aug 2011 was performed and cross-referenced. Inclusion criteria: 1)Patients with diagnosis ALF, ACLF or hyperammonemia without liver failure, 2) Brain MRI was performed with diffusion weighted imaging (DWI) sequences, 3) description of

neurological outcome. All de-identified imaging was reanalyzed by an attending neuroradiologist. 36 (28 adults, 8 pediatric) patients from 11 peer authored publications were analyzed together with data collected retrospectively from 9 (8 adults and 1 pediatric) of our patients. 25 presented with ACLF, 9 with ALF and 11 with isolated hyperammonemia form heterogeneous etiologies.
Results: The indication for MRI was for evaluation of coma in all patients. Amongst the 53% (24) of patients with severe hyperammonemia (plasma ammonia level (PAL) > 150 µmol/L), 18 patients had diffuse cortical restricted diffusion (DCRD) on MRI, whilst, outcome A, B, and C occurred respectively in 6, 3 and 17 patients. Of the 47% (21) of patients with DCRD on MRI irrespective of diagnosis or PAL, outcome A, B and C occurred respectively in 2, 3 and 16 patients. Independent predictors associated with poor outcome include the presence of DCRD ($p<0.0009$) and severe hyperammonemia ($p = 0.0008$). Absence of DCRD was also a strong predictor for good outcome ($p< 0.0001$).
Conclusion: MRI may provide an important utility in assessing patients with hepatic encephalopathy especially in the setting of severe hyperammonemia.

O17 BRAIN MAGNETIC RESONANCE IN EPISODIC HEPATIC ENCEPHALOPATHY.

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Background: Brain magnetic resonance (MR) has shown a series of metabolic abnormalities and changes in the amount and distribution of water that could be useful for the diagnosis of hepatic encephalopathy. However, most MR studies have been performed in patients with minimal HE and few studies have reassessed the same patient after the recovery of HE. Furthermore, MR equipments lacked enough resolution to separate glutamine and glutamate peaks.
Aim of the study: to investigate the disturbances of brain water and metabolites and relate them to the outcome of HE using a 3T-MR scanner.
Methods: Cirrhotic patients with overt signs of HE (n=18) at the time of admission (grade I-II: n=6, grade III-IV: n=12) and a group of healthy voluntaries (n=8) underwent MR assessment that was repeated at six weeks, after resolution of HE (n=14).
Results: Brain glutamine was high at baseline (Gln/Cr: 2.40±0.78 vs 0.22±0.08; $P<0.001$), decreased during follow-up (1.55±0.55; $P=0.028$), related to the severity of HE ($r=0.62$; $P=0.006$) and correlated to plasma ammonia ($r=0.513$; $P=0.006$). During HE patients exhibited high apparent diffusion coefficient compatible with vasogenic edema that decreased when patients recovered from HE (corticospinal tract: from 780±44 µm2/s to 758±44 µm2/s; $P=0.025$; parietal white matter: from 884±54 µm2/s to 842±38 µm2/s; $P=0.016$). However, apparent diffusion coefficient values did not correlate to the grade of HE.
Conclusions: Brain glutamine assessed by MR-spectroscopy could be a good biomarker for the assessment of HE. The increase of water in HE is limited to the extracellular compartment suggesting vasogenic edema secondary to disturbances in the blood-brain-barrier. However, the pathogenesis of HE does not seem to be directly imputable to brain edema.

SESSION 5: HYPERAMMONEMIA: RATIONALE FOR TREATMENT

O18 SYSTEMATIC REVIEW OF RANDOMISED CONTROLLED TRIALS ON ORAL BRANCHED-CHAIN AMINO ACIDS FOR RECURRENT HEPATIC ENCEPHALOPATHY

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We systematically reviewed the effects of oral branched-chain amino acids (BCAA) for patients with recurrent hepatic encephalopathy (HE). Randomized controlled trials on oral BCAA versus control supplements or placebo. Trials were identified through electronic and manual searches were included. Data were retrieved through correspondence with the authors of included trials and published reports. Random effects meta-analyses, subgroup, meta-regression regression (Egger's test) and sequential analyses were performed with results presented as risk differences (RD) with 95% confidence intervals (CI). RESULTS: Eight randomized controlled trials with a total of 382 patients were Included patients were diagnosed as having cirrhosis and clinically overt HE (four trials) or minimal HE (four trials). Individual patient data were available for the four largest trials (255 patients). Trial level data were available for the remaining four trials (127 patients). The mean daily dose of the oral BCAA supplements was 0.25 g/kg. Random effects meta-analysis showed that BCAA improved manifestations of He compared with control diets or placebo (87 of 172 versus 56 of 210 patients; RD 0.21[0.09-0.34]; number needed to treat 5 patients). The result was confirmed in analyses stratified by bias control and sequential analyses adjusting for multiple comparisons. No evidence of publication bias or other biases were seen in regression analysis. The effect of BCAA was different (P=0.05) in clinically overt compared with minimal HE (RD 0.30 [0.16-0.44] and RD 0.10 [-0.05 to 0.25]). No other predictors of intertrial heterogeneity were identified. BCAA supplements had no effect on mortality (RD -0.01[-0.07-0.04) or markers of nutritional status. The frequency of adverse events was similar in the BCAA and control groups. CONCLUSION: Oral BCAA supplements improve the manifestations of recurrent HE, but have no effect on survival.

O19 ORNITHINE-PHENYLACETATE REVISITED

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Over the past 100 years a central role of ammonia in the pathogenesis of hepatic encephalopathy has been hypothesised. Treatment of hyperammonemia and hepatic encephalopathy are unmet clinical needs. Ornithine phenylacetate (OP) is a novel drug that is targeted at reducing ammonia concentration in patients with liver disease and therefore a potential treatment for hepatic encephalopathy (HE). The mechanisms include ammonia-induced changes in neurotransmitter synthesis and release, neuronal oxidative stress, impaired mitochondrial function and osmotic disturbances resulting from astrocytic metabolism of ammonia to glutamine. Systemic hyperammonemia has been largely found in patients with HE with underlying cirrhosis and acute liver failure (ALF). The mechanism by which OP directly reduces ammonia levels in cirrhosis is by increasing muscle glutamine synthesis activity, subsequently trapping and increasing

ammonia excretion as phenylacetylglutamine, with the concomitant normalization of gut glutaminase activity. Recent studies suggest that superimposed infection or inflammation on the background of hyperammonemia worsens the severity of hepatic encephalopathy. In fact, hyperammonemia itself has been shown to produce failure of neutrophil function and also induce neuro-inflammation. Studies in animal models indicate that the reduction of ammonia levels in animal models is associated with a reduction in systemic and brain inflammation which also improves brain nitric oxide availability in cirrhotic patients. These pathophysiological changes induced by OP have been shown to reduce brain edema in cirrhotic animals, and a reduction in intracranial hypertension in a pig model of ALF. In portacaval shunted animals, OP prevented neurophysiological functional deterioration precipitated by induced hyperammonemia. Studies to date have indicated that it is safe and patient studies in minimal HE and trials in overt HE are underway to establish OP as a treatment for this major complication of liver disease.

O20 L-ACETYLCARNITINE IN HEPATIC ENCEPHALOPATHY

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Hepatic encephalopathy (HE) is a common complication of hepatic cirrhosis. The clinical diagnosis is based on two concurrent types of symptoms: impaired mental status and impaired neuromotor function. Impaired mental status is characterized by deterioration in mental status with psychomotor dysfunction, impaired memory, and increased reaction time, sensory abnormalities, poor concentration, disorientation and coma. Impaired Neuromotor function includes hyperreflexia, rigidity, myoclonus and asterixis. The pathogenesis of HE has not been clearly defined. The general consensus is that elevated levels of ammonia and an inflammatory response work in synergy to cause astrocyte to swell and fluid to accumulate in the brain which is thought to explain the symptoms of HE. Various authors demonstrated that L-carnitine represent an osmoprotectant compound and act on ammonia decrease. Acetyl-L-Carnitine (ALC) is a carnitine derivative. Various studies suggest a beneficial effect in patients with minimal, mild, moderate, and severe hepatic encephalopathy. ALC administration has shown the recovery of neuropsychological activities related to attention/concentration, visual scanning and tracking, psychomotor speed and mental flexibility, language short-term memory, attention, and computing ability. In fact, ALC induces ureagenesis leading to decreased blood and brain ammonia levels. ALC treatment also improves health-related quality of life and physical activity, and decreases the severity of mental and physical fatigue.

O21 PROTEIN MALNUTRITION INCREASES THE RISK OF OVERT AND MINIMAL HEPATIC ENCEPHALOPATHY

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Protein malnutrition frequently occurs in cirrhotic patients and could increase the risk of several complications, such as variceal bleeding, ascites, hepatic encephalopathy (HE), hepatorenal syndrome and infections. On the other hand, protein-restricted diets are frequently prescribed to cirrhotic patients with HE leading to a further deterioration of the nutritional status increasing protein catabolism and the release of aminoacids from the

muscle. The influence of protein malnutrition on minimal HE(MHE) has not been investigated. Our study was aimed to verify the relationship between protein malnutrition and the prevalence of overt HE and MHE in a large cohort of cirrhotic patients.
We enrolled consecutive cirrhotic patients without known neurological diseases referred to our University Hospital. Protein malnutrition was diagnosed based on anthropometry (Mid-Arm Muscle Circumference <5thpercentile). A pool of standardized questions, evaluating the time and space orientation, was used to diagnose HE. Three paper and pencil tests (TMT-A, TMT-B and DST) were used for the diagnosis of MHE.
Two-hundred-five cirrhotic patients (age 62±13 years; 56%Child B-C) were enrolled. Forty-three patients (21%) showed an episode of HE at admission or during the hospitalization. Furthermore 21 patients (10%), without overt HE during their hospital stay, reported previous episodes in their clinical history. One-hundred-two patients without HE were evaluated for MHE: a diagnosis was made in 36 patients (35%). Protein malnutrition was detected in 98 patients. The prevalence of malnutrition was higher in patients who experienced HE vs those with no history of HE (59% vs 42%; p=0.04). Similarly, protein malnutrition was more frequent in patients with MHE vs those without MHE (51% vs 31%; p<0.042).
Our study shows that protein malnutrition is a risk factor for HE and MHE suggesting that the amelioration of nutritional status may decrease the prevalence of subclinical alterations of mental status in cirrhotic patients.

O22 METFORMIN PROTECTS AGAINST HEPATIC ENCEPHALOPATHY: IMPACT ON INSULIN RESISTANCE AND GLUTAMINASE ACTIVITY

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BACKGROUND: Studies have related cirrhosis outcomes, including hepatocellular carcinoma and hepatic encephalopathy (HE), to type 2 diabetes mellitus (T2DM). The intestinal production of ammonia depends on the action of the colon bacteria and the glutaminase activity, which plays a major role in the pathogenesis of HE.
AIM: To assess the impact of metformin use on liver dysfunction and HE in cirrhotic patients and the mode of action implicated on.
METHODS: Two-hundred-and-twenty-eight patients were included: 47.4% (108/228) alcoholic cirrhosis, 36% (82/228) HCV-related and 16.6% (38/228) others; 163 males and 65 females, with mean age of 56.8±10.8 years. Baseline Child-Pugh 6.3.±1.6 and MELD 10.4±4.2. Diabetic patients were classified as insulin sensitizers experienced (metformin with or without pioglitazone) (n=41); cirrhotics with insulin-dependent T2DM (n=28); and diabetics with dietetic treatment (n=13). Follow-up mean time was 40.6±26.9 months. Baseline analysis included: insulin, glucose, glucagon, leptin, adiponectin, TNFr2, AST, ALT. HOMA-IR was calculated. End-points were the relationship between HE and metformin use and HE and HOMA-IR.
RESULTS: Hepatic encephalopathy was observed in 24.1% (55/228): 28.3% (53/187) in patients who did not receive metformin and 4.8% (2/41) in patients treated with metformin. In multivariate analysis, metformin use [H.R.6.79 (95%CI: 1.65-28.04);p=0.008], age at diagnosis [H.R.1.06 (95%CI: 1.03-1.09);p=0.001] and Child-Pugh [H.R.1.54 (95%CI: 1.34-1.77);p=0.001] were found independently associated with HE. HOMA-IR>4 showed more HE events than HOMA-IR<4 (28.3% vs 14.7%; Log Rank 4.8; p=0.028). In vitro, metformin use decreased the glutaminase activity up to 82.4% with a concentration of 10mM and to 32% with 100mM (1mM is 875mg of metformin). CONCLUSIONS: Metformin was found independently related to overt hepatic encephalopathy, which could be explained, at least in part, by glutaminase activity inhibition and improving of insulin resistance.

SESSION 6: AMMONIA AND CEREBRAL ENERGY METABOLISM

O23 EFFECTS OF AMMONIA ON ENERGY METABOLISM AND SYNTHESIS OF NEUROTRANSMITTER GABA IN MOUSE CEREBRAL CORTICAL CELL CULTURES.

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Co-cultures of mouse cortical neurons and astrocytes when exposed to high levels of ammonia responded with increasing neuronal TCA cycle activity and also switching the astrocytic TCA cycle toward synthesis of substrate for glutamine synthesis. In addition, ammonia up-regulated glycolysis in both co-cultures of GABAergic neurons and astrocytes and cultures of GABAergic neurons. Particularly, only the energy substrate glucose was able to sustain the cellular energy metabolism in the form of ATP synthesis. Ammonia also altered the profile of neurotransmitter GABA synthesis in co-cultures of cortical neurons and astrocytes. It was observed that more GABA was synthesized via the indirect pathway, which utilizes precursors from the TCA cycle, relative to the direct pathway, in which glutamate undergoes decarboxylation directly to GABA. Interestingly, this switch in GABA synthesis was also observed in the cerebral cortex of bile duct-ligated rats. Since the indirect pathway is associated with synthesis of vesicular GABA, this might explain the increased GABAergic tone in hepatic encephalopathy.

O24 AMMONIA AND OXIDATIVE STRESS

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There is ample amount of evidence that suggests oxidative stress plays a pivotal role in the pathogenesis of hepatic encephalopathy (HE). Increased levels of reactive oxygen/nitrogen species (ROS/RNS), such as superoxide anion (O_2^-), hydroxyl radical (^-OH) and peroxynitrite ($ONOO^-$) can cause significant damage to major components of the cell; proteins (affecting activity and expression), nucleic acids (RNA oxidation) and lipids (peroxidation of membrane lipids). It has been demonstrated that cerebral ammonia toxicity involves the generation of ROS/RNS. *In vitro* studies have shown that cultured astrocytes treated with an acute dose of ammonia (1-5mM) leads to the production of ROS. Furthermore, portacaval shunted (PCS) rats injected with an acute dose of ammonia develop cerebral oxidative stress in association with severe HE and death. However, in the context of chronic hyperammonemia, our group have not detected any signs of oxidative stress in the brains of either PCS or bile-duct ligated (BDL) rats. Instead, we found that systemic, and not central, oxidative stress is an important player in exacerbating the neuropsychological effects of hyperammonemia. An increase in ROS in the blood is believed to arise from a disturbance in the liver's redox state during liver disease leading to an imbalance between the production of ROS and antioxidant capacity. We have recently demonstrated chronic hyperammonemia independently does not lead to an increase in brain water. When BDL rats are treated with allopurinol (xanthine oxidase inhibitor) to attenuate plasma ROS, brain edema is reduced. However, systemic oxidative stress also independently does not induce brain

edema in BDL rats since when BDL rats were treated with AST-120 (activated carbon microspheres) which lead to an attenuation blood ammonia with no change in systemic ROS, a decrease in brain edema was observed. Overall, this implies systemic oxidative stress and hyperammonemia synergistically induce brain edema.

O25 HEPATIC ENCEPHALOPATHY IS ASSOCIATED WITH DECREASED BRAIN OXYGEN METABOLISM AND BLOOD FLOW, NOT INCREASED AMMONIA UPTAKE

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Background and aims: It is unknown whether the reduced oxygen metabolism and cerebral blood flow (CBF) in cirrhotic patients with hepatic encephalopathy (HE) relate to the HE or the liver disease, and whether the impaired brain oxidative metabolism is related to the high blood ammonia. We studied these questions in a paired study.
Methods: We measured cerebral oxygen metabolism, CBF, and brain ammonia uptake by dynamic PET/CT in nine cirrhotic patients during an episode of overt HE type C, and again after recovery. Nine patients with cirrhosis with no history of HE were controls.
Results: The mean cerebral oxygen metabolism was 0.73 µmol oxygen/mL brain /min during HE and rose to 0.91 after recovery ($P < 0.05$). The CBF was 0.28 mL blood/mL brain/min during HE and rose to 0.38 after recovery ($P < 0.05$). Both recovery values were similar to the control values. Blood ammonia decreased by 20% after recovery ($P < 0.05$) whereas brain ammonia uptake did not decrease ($P > 0.30$).
Conclusions: The decreased cerebral oxygen metabolism and CBF during the episode of HE were reversible and thus associated to the HE rather than the liver disease as such. Moreover, the similar cerebral ammonia uptake during and after HE does not support a primary toxic effect of ammonia on brain oxidative metabolism.

O26 INCREASED CEREBRAL LACTATE CONTRIBUTES TO BRAIN EDEMA IN CIRRHOTIC RATS

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Aims: Neurotoxic effects of ammonia have been shown to induce numerous metabolic alterations such as increased brain glutamine and lactate. In brain edema associated with acute liver failure, glutamine plays a controversial role and a few studies demonstrated lactate is also involved. Brain edema is also present in patients with chronic liver failure; in this situation roles of lactatae and glutamine are less understood. We previously demonstrated an increase in lactate de novo synthesis in bile-duct ligated rats (BDL), a known model of chronic liver failure/minimal hepatic encephalopathy. To define the role of lactate in the pathogenesis of brain edema, the present study investigates the effects of inhibiting lactate production.
Methods: BDL rats were treated with dichloroacetate (DCA, 25 mg/kg for 7 days starting at week 5 after intervention, intraperitoneally), a pyruvate dehydrogenase kinase inhibitor that leads to pyruvate dyhydrogenase stimulation, therefore to a shift of pyruvate from glycolysis to oxidation and consequently to a decrease in lactate production. Brain edema (specific gravimetric technique) and glutamine (HPLC) were measured in brain tissue of BDL rats vs sham operated controls.

Results: 6 weeks following BDL, rals develop brain edema and increased brain lactate compared to SHAM-operated controls. DCA treatment normalized brian lactate in BDL rats. DCA-treated BDL rats demonstrated a significant reduction of brain water content reaching values similar to those sseen SHAM-operated rats. Glutamine levels were increased in BDL vs SHAM operated rats and remained high in DCA-treated BDL rats (569.20±80.44 µM vs BDL: 796.60±71.50 µM, and vs SHAM: 442.80±33.79 µM). Conclusions: Inhibition of lactate production attenuated brain edema. Impaired lactate metabolism contributes to the pathogenesis of brain edema in minimal hepatic encephalopathy.

SESSION 7: CEREBRAL AMMONIA CONVERSION

O27 POSSIBLE TREATMENT OF END-STAGE HYPERAMMONEMIC ENCEPHALOPATHY BY INHIBITION OF GLUTAMINE SYNTHETASE

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Extrahepatic tissues rely on glutamine synthetase (GS) [Glu + NH3 + ATP _ Gln + ADP + Pi] to remove circulating and metabolically produced ammonia. Although there is little doubt that excess ammonia promotes neurotoxicity in liver disease patients, circulating levels may vary considerably, and hepatic encephalopathy often correlates poorly with blood ammonia. However, elevated glutamine is an indicator of prolonged, but fluctuating hyperammonemia. It is now accepted that overproduction of glutamine contributes, at least in part, to hyperammonemic encephalopathy. In the brain, GS is highly active in astrocytes, and these cells are physiologically and morphologically compromised by hyperammonemia. Hyperammonemia in end-stage acute liver failure (ALF) is associated with cerebral edema and astrocyte pathology/swelling. Many animal studies, and more recently non-invasive magnetic resonance studies of liver disease patients, have shown that cerebral glutamine concentrations are greatly elevated by hyperammonemia, whereas there is less change in glutamate. The mechanism whereby excess glutamine is neurotoxic is the subject of ongoing debate. Nevertheless, treatment of end-stage ALF patients with a GS inhibitor might reduce the potentially fatal cerebral edema. Many animal studies have shown that the GS inhibitor L-methionine-*S*,*R*-sulfoximine (MSO) is protective against acute ammonia-induced intoxication. MSO is also an inhibitor of glutamate cysteine ligase, can be converted to metabolic products *in vivo*, and causes convulsions at high doses. However, the susceptibility to MSO-induced convulsions is species dependent, with primates being relatively resistant. Moreover, it is possible to chronically maintain cerebral GS activity in mice at low levels by MSO treatment without any obvious untoward effects. Apparently, convulsions occur only when a threshold is exceeded, such that brain GS is almost totally inhibited. Extreme caution would be needed in administering MSO to patients. Nevertheless, inhibition of brain GS by MSO (or a non-metabolizable, more selective analogue) may have therapeutic benefit in end-stage ALF.

O28 ALANINE AS AN ALTERNATIVE AMMONIA SCAVENGER

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When subjected to high levels of ammonia, co-cultured neurons and astrocytes synthesizes not only glutamine but also alanine, the latter process involving the concerted action of glutamate dehydrogenase (GDH) and alanine aminotransferase (ALAT). The glutamine synthetase (GS) inhibitor methionine sulfoximine enhances synthesis and release of alanine in co-cultures by blocking the GS-dependent ammonia scavenging process underlining the importance of the GDH-ALAT biosynthetic pathway for ammonia fixation. Provided that the brain toxicity and edema induced by

hyperammonemia are related to glutamine synthesis, this points to the use of a GS inhibitor in treating the deleterious cerebral effects of elevated blood ammonia levels.

O29 DOES INHIBITION OF GLUTAMINE SYNTHESIS INDUCE GLUTAMATE DEHYDROGENASE-DEPENDENT AMMONIA FIXATION INTO ALANINE IN AN *IN VIVO* RAT MODEL OF HYPERAMMONEMIA?

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Background: Hyperammonemia is believed to play an essential role in the pathogenesis of hepatic encephalopathy (HE) and it has been shown that brain glutamine concentration during episodes of HE is increased, suggesting that elevated brain glutamine causes the symptoms. Glutamine is formed from glutamate catalyzed by glutamine synthetase (GS) and the process can be blocked by methionine sulfoximine (MSO). The aim of this study was to investigate how ammonia is detoxified in the brain and other tissues when GS is inhibited by MSO.
Materials and Methods: Female Wistar rats were subjected to bile duct ligation (BDL) or SHAM operation. Six weeks later half of each group received an injection of either MSO or saline and 3 hours later at the end of a 15 minute intravenous infusion of [^{15}N]ammonia, arterial blood was withdrawn. Immediately after the rats were decapitated and samples of brain, liver, kidney and muscles were collected. The incorporation of labeled ammonia in glutamate, glutamine, alanine, aspartate and concentrations of these amino acids were analyzed in blood and tissue samples by GC-MS and HPLC.
Results: BDL and SHAM rats had similar labeling patterns and concentrations of amino acids in plasma and all tissues examined, although BDL rats had higher arterial blood ammonia ($P<0.05$). In the following the results from the BDL rats are shown, where MSO (n=10) is compared to NaCl (n=10). In the brain, treatment with MSO led to a decrease in single labeled glutamine (NaCl 24.9% ± 1.4 vs. MSO 12.1% ± 1.4, $P<0.001$) and increase in labeled alanine (NaCl 0.2% ± 0.1vs. MSO 5.5% ± 1.0, $P<0.001$). In plasma, liver, kidney and muscle single labeled glutamine were also decreased ($P<0.001$) when treated with MSO, but alanine labeling was only increased in plasma ($P<0.05$). The content of glutamine was unchanged in brain and muscle ($P>0.1$) but was increased in liver, kidney and plasma ($P<0.001$) in the MSO group. Alanine content was raised in plasma and brain ($P<0.01$). **Conclusion**: The results show that brain ammonia is trapped in alanine when glutamine synthesis is blocked by MSO and hence alanine synthesis can act as an ammonia detoxifying process in the brain. MSO did not change the alanine synthesis in other tissues.

O30 THE EFFECT OF AMMONIUM ON EXTRACELLULAR LACTATE, ADENOSINE AND GLUTAMATE IN CORTEX OF RAT BRAIN SLICES. A BIOSENSOR DOSE-RESPONSE STUDY.

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Hyperammonemia is thought to be associated with alterations in cerebral oxidative metabolism and glutamatergic neurotransmission. Our understanding of cerebral

pathophysiology related to hyperammonemia is based on studies of cell cultures, animal models and clinical studies where the actual tissue or plasma concentrations of ammonium range from clinical relevant levels in the micromolar range to more than 5 mM. To assess the significance of the ammonium concentration we studied the extracellular release of lactate, adenosine and glutamate in cerebral cortex of rat brain slices in a dose-response study. We applied concentrations of ammonium from 0.15 mM to 10 mM to 29 brain slices in a perfusion chamber with exposure times up to 90 minutes. We measured the extracellular changes in lactate, adenosine and glutamate by the use of biosensors inserted into cerebral cortex. We found a consistent reduction in the extracellular lactate concentration ranging from 4 to 400 micromolar independent of the ammonium concentration ($R2=0.006$, ammonium vs. lactate). The reduction in lactate was not affected by inhibition of the neuronal lactate transporter MCT-2 by adding alpha-cyano-4-hydroxycinnamic acid. We found a positive correlation between the ammonium concentration and the change in adenosine ($R2=0.68$, $p<0.05$) where ammonium levels above 500 mM were associated with adenosine release up to 18 micromolar. Finally, we observed a positive correlation between the ammonium level and glutamate increase ($R2=0.43$, $p<0.05$) with a marked release of glutamate (up to 55 micromolar) with exposure to 10 mM of ammonium. In conclusion, cortical tissue exposed ammonium displayed a linear dose-response-like relationship between ammonium concentration and the changes in adenosine and glutamate. Interestingly, we found that ammonium induced a reduction in the extracellular lactate concentration independent of the ammonium concentration within the studied range. The reduction appeared not to be related to increased neuronal uptake of lactate.

SESSION 8: MINIMAL HEPATIC ENCEPHALOPATHY

O31 PSYCHOMETRIC TESTS FOR MINIMAL HEPATIC ENCEPHALOPATHY

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Abnormal psychometric test results and/or neurophysiological findings indicating brain dysfunction are considered diagnostic for minimal hepatic encephalopathy (mHE) in patients with liver cirrhosis and no clinical signs of HE. Various tests and test batteries have been used for the diagnosis of mHE over the past four decades, competitively, and there is still no consensus which one should be the first choice or "gold standard". Several of the single tests applied only assess aspects of the attention system – some just vigilance, others only higher executive function. The test batteries used so far in addition include sub-tests for memory, verbal and motor function.
Discussions about the most appropriate diagnostic method for the assessment of HE circle around simplicity, time and personnel needed to perform the test more than sensitivity and specificity for diagnosing mHE. Although it is well known that brain dysfunction in patients with mHE is multifaceted there is still the desire for the one and only simple method that is able to detect mHE without any doubt. In fact psychometric testing for mHE should consider all cognitive domains that may be affected – that would be attention, psychomotor speed, motor accuracy, and visuo-spatial abilities – and should take into account that none of the measures applied is able to make the diagnosis mHE beyond doubt. Psychometric tests offer insight into a subjects' actual cognitive performance but are indifferent against the agent behind cognitive dysfunction.

O32 COGNITIVE IMPAIRMENTS AND HEPATIC ENCEPHALOPATHY

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Hepatic encephalopathy (HE) is a frequent and severe complication of cirrhosiswith a incidence lower than that of ascites but much more higher than that of variceal bleeding. HE is a complex neurological syndrome characterized by a wide spectrum of neuropsychiatric changes and alterations in neuromuscular function. It may be overt or minimal. The latter refers to the occurrence of subclinical alterations detectable only by psychometric or electrophysiological techniques. HE negatively affects the patient's self sufficiency and the quality of life. Even minimal HE has been shown to reduce the quality of life and to negatively affect the patients' manual ability and driving capacity. MHE affects up to 20-60% of patients with cirrhosis, depending on the methods utilized for its diagnosis and on the severity of underlying liver disease and have important clinical implications, being associated to a reduced quality of life. Moreover, MHE impairs the execution of simple and complex tasks and, in the recent years, has been repeatedly associated to an increase number of car accidents and traffic violations. Finally, MHE may have a prognostic value for survival and is considered a risk factor for the development of episodes of overt HE. It has been recently observed that even a single episode of OHE is accompanied by a persistent cognitive defect thus suggesting that HE cannot be considered a fully reversible condition. Finally MHE has been associated to falls in patients with cirrhosis.
Although the neuropsychological characterization of MHE is not yet perfect, the main features of the MHE are alterations of psychomotor speed and of executive functions, in particular of the anterior attentive system, even though memory dysfunctions have been inconstantly detected. To avoid problems related to the use of psychometric test batteries, namely the need of a dedicated personnel, some other diagnostic tools have been proposed: the critical flicker frequency, the smooth pursuit eye movements, the cognitive evoked potentials and two more specific and easy-to-repeat computerized

psychometric tests: the Scan test and the inhibitory control test. In addition to the above-mentioned techniques, also the EEG is a highly effective in diagnosing MHE, mainly when basal activity is quantified.
The optimal diagnosis of MHE is still a matter of debate. The Psychometric Hepatic Encephalopathy Score (PHES), a paper-and-pencil test battery, which includes Digit-Symbol Test (DST), Trail-Making Test A, Trail-Making Test B, Serial-Dotting Test (SDT) and Line-Tracing Test (LTT) is the tool suggested by a consensus report on the diagnosis and quantification of HE. Nevertheless, PHES is rarely used. In our opinion, a major clinically relevant interest in searching for MHE is the possibility of identifying those patients with cirrhosis who have a tendency to develop an overt episode of HE during the follow up. In these patients, prophylactic treatments may be administered and those procedures known to be associated to the development of HE, such as TIPS, may be avoided. Moreover, in the last few years, a novel approach in testing the efficacy of drugs for HE has been proposed. Randomized controlled trials have been carried out by enrolling cirrhotic patients at risk of HE but without any evidence of HE at inclusion. In these studies, the occurrence of any episode of overt HE represents a more objective end-point than the amelioration of HE symptoms, being the resolution of HE influenced by a number of factors such as the resolution of the precipitating event, the correction of anaemia and electrolytes imbalance, etc. Until now, these prophylactic studies have enrolled post-TIPS patients or those who have already experienced one or more episodes of overt HE. Another category of patients at risk of overt HE is however represented by those with a MHE identified according to the psychometric evaluation of the critical flicker frequency and to an altered EEG on quantitative evaluation.

O33 CIRCADIAN RHYTHMS IN PATIENTS WITH CIRRHOSIS

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Circadian sleep regulation is responsible for the alternation of periods of high/low sleep propensity, in relation to dark/light cues, and irrespective of preceding sleep-wake behaviour. This process is marked by the hormone melatonin, which is high at night and low in the daytime hours, when its cerebral synthesis is suppressed by light (1). As melatonin is metabolised by the liver, it has been assumed that its disposition would be impaired in patients with cirrhosis. Abnormalities have been observed to support this contention: these include high daytime melatonin concentrations (2), low concentrations of the urinary metabolite 6-sulphatoxymelatonin (aMT6s)(3), and a reduction in the clearance of exogenously administered melatonin (4). In one study where endogenous plasma melatonin and urinary aMT6s were assessed simultaneously, overnight melatonin clearance was found to be reduced (5). In the same and other studies, correlations were observed between the delay in plasma melatonin/urinary aMT6s peaks and the degree of hepatic failure (5-7). However, other circadian abnormalities have also been described, to include delays in the nocturnal rise of melatonin and in its time to peak (2,7), suggesting dysfunction of the central circadian clock. Montagnese and co-workers demonstrated parallel delays in the onset of plasma melatonin/cortisol rhythms and attenuated melatonin sensitivity to light in a group of 20 patients with cirrhosis, also indicating central circadian dysfunction (5). Some attempt has been made to correlate the changes in the melatonin rhythm with the sleep disturbances observed in patients with cirrhosis, but the findings have been inconclusive (5,8). It has been suggested that circadian rhythm delays in this patient population are associated with delayed sleep habits, although not necessarily with impaired sleep quality (6). One encouraging case report suggests that treatment with light may help correcting these timing abnormalities (9).
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O34 CORRELATION BETWEEN DEGREE AND QUALITY OF SLEEP DISTURBANCE AND THE LEVEL OF NEUROPSYCHIATRIC IMPAIRMENT IN PATIENTS OF CIRRHOSIS

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Background and Aim: Sleep disturbances are common in patients of cirrhosis and have a significant effect on their health-related quality of life (HRQOL). Thus far, no study has demonstrated a systematically studied significant correlation between the sleep disturbance observed and the neuropsychiatric impairment status of patients of cirrhosis.
Method: On the basis of psychometric hepatic encephalopathy score (PHES), we divided 100 cirrhotic patients into those having minimal hepatic encephalopathy (MHE) (PHES ≤ -5) and those not having (NMHE). Now, in these MHE (n = 46) and NMHE (n = 54) patients, sleep disturbance was measured with Pittsburgh Sleep Quality Index (PSQI) and Epworth Sleepiness Scale (ESS) and HRQOL with SF-36 (v2) questionnaire.
Result: Sixty (60%) patients were found to be 'poor sleepers' (PSQI > 5) while 38 (38%) patients had excessive daytime somnolence (EDS) (ESS ≥ 11). Univariate and multivariate analyses showed MHE status of the patients to have significant effect among 'poor sleepers' ($P < 0.0001$) as well as on those with EDS ($P < 0.0001$). Significant correlation existed between PHES and both the sleep parameters of PSQI ($r = -0.518$, $P < 0.0001$) as well as ESS ($r = -0.383$, $P < 0.0001$), implying independently strong correlation between MHE status and the presence of night-time sleep disturbance and daytime somnolence among cirrhotic patients. Significant correlation existed between PSQI and ESS and the various scales and component scores of SF-36 (v2) signifying the negative impact of sleep disturbance on the HRQOL.
Conclusion: Both night-time sleep disturbance and EDS have significant relation with the neuropsychiatric impairment in patients of cirrhosis and are significantly associated with the observed impairment in HRQOL.

O35 FLICKER FUSION FREQUENCIES FOR THE DIAGNOSIS OF HEPATIC ENCEPHALOPATHY: CONFOUNDING VARIABLES AND BEST PERFORMANCE MEASURES

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Introduction: There is no gold standard for the diagnosis of hepatic encephalopathy (HE). Measurement of flicker fusion frequencies appears to hold promise. The aim of this study was to identify best performance measures and potential confounding variables.

Methods: Eighty patients with cirrhosis (49 men, 31 women; mean [range] age 59 [32-81] yr) were classified, using clinical, psychometric and electrophysiological variables, as neuropsychiatrically unimpaired (n=43), or as having minimal (n=19) or overt (n=18) HE. A further 66 patients (44 men, 22 women; mean age 55 [34-75] yr) were recruited for validation. Forty healthy individuals (17 men, 23 women; mean age 40 [21-70] yr) served as controls. The Lafayette model 12021 was used to measure flicker and fusion frequencies and their mean, the critical flicker fusion (CFF) frequency; data were age- and sex-adjusted.

Results: Fusion and CFF frequencies distinguished the population subgroups: viz. fusion frequency (mean±1SD): controls (36.6±2.5 Hz) vs. unimpaired patients (34.1±3.1 Hz) vs. minimal (30.3±4.2 Hz) vs. overt HE (25.9±7.8 Hz) ($p<0.0001$). A fusion frequency threshold of 33.4 Hz had a diagnostic sensitivity of 78.8% and a specificity of 78.3% for any degree of HE; disease-specific thresholds performed better viz. alcohol-related cirrhosis: threshold 32.9 Hz, sensitivity 80.0%, specificity 81.8%; non-alcohol related cirrhosis: threshold 33.6 Hz, sensitivity 76.9%, specificity 91.3%. The diagnostic utility of these thresholds was confirmed in the validation population. A CFF frequency of <33.3 Hz predicted death/liver transplantation, independently of the degree of hepatic decompensation (hazard ratio 3.34, 95% confidence interval 1.35-8.27; $p=0.009$).

Conclusion: Fusion and CFF frequencies have significant potential for the diagnosis of HE and for predicting outcome.

O36 THE UTILITY OF EVOKED POTENTIAL FOR THE DIAGNOSIS OF HEPATIC ENCEPHALOPATHY—IT'S ALL IN THE PROCESSING

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Introduction: Various EP paradigms have been utilized for the diagnosis of hepatic encephalopathy (HE) but, with the exception of the cognitive P300, the results have been inconsistent and hence the techniques are not generally utilized. In previous studies signal analysis was confined to measurements in the time-domain. The aim of this study was to apply frequency-domain processing of EPs in patients with cirrhosis in relation to their neuropsychiatric status

Methods: Seventy patients with cirrhosis (47 men: 23 women; mean age 55.1 [37–78] years) were classified, using clinical, psychometric and EEG criteria as: unimpaired (n=27; 38.6%) or as having minimal (n=13; 18.6%) or overt (n=30; 42.6%) HE. Forty-

eight healthy subjects (25 men: 23 women; mean [range] age 39.8 [22–68] years) served as controls. Visual (VEPs), brainstem auditory (BAEPs) somatosensory (SSEPs) and P300 cognitive auditory EPs were recorded under standardized conditions, in a single sitting. EP signals were processed using Power Spectral Density Estimates (PSD). Results: Conventional signal processing identified significant differences in EPs between controls and patients but few if any significant differences in the patient subgroup. In contrast frequency domain processing better differentiated the patient subgroups (Table 1).

Table 1: EP peak frequencies in controls and patients with cirrhosis, by neuropsychiatric status

Evoked Potential	Controls (n = 17)	Unimpaired (n=26)	Minimal HE (n= 12)	Overt HE (n=12)
VEP				
$F_{(peak)}$ Hz	12.1±3.9	11.1±4.2°°+++	8.0±4.6***	6.1±3.5***
SSEP				
$F_{(peak)}$ Hz	69.7±14.0	41.8±28.6**°	25.7±13.5***	30.7±27.4***
BAEP				
$F_{(peak)}$ Hz	174.9±83.9	152.4±81.1°°°++	87.4±36.0***	84.06±20.5****
P300 Auditory Cognitive				
$F_{(peak)}$ Hz	7.7±2.6	5.9±2.3*°°°+++	3.8±1.5****+	2.6±0.9****

Mean± SD
Differences from controls: $^{*}p < 0.05$, $^{**}p <0.005$, $^{****}p <0.001$, $^{*****}p <0.0001$
Differences from: minimal HE: $^{°}p <0.01$, $^{°°}p <0.005$, $^{°°°}p <0.0005$
Differences from overt HE: $^{+}p < 0.05$, $^{++}p <0.01$, $^{+++}p <0.0005$

Conclusion: Frequency-domain analysis of EPs should be used to compliment time-domain analysis in patients with HE.

SESSION 9: POSTER SESSION

See poster section in program for abstracts.

SESSION 10: GUT-LIVER-AXIS: RATIONALE FOR TREATMENT

O37 IMPORTANCE OF GUT FLORA IN HE - NEW RESULTS

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The pathogenesis of hepatic encephalopathy (HE) remains incompletely understood; Ammonia, which is primarily produced in the gut, plays a key role in the pathogenesis of HE. Glutamine deamination in small-intestine mucosa and urease-containing species, such as *Klebsiella* and *Proteus* species in the intestine are the major source of ammonia production. Recent evidences suggest that inflammation plays a synergistic role with ammonia in producing and modulating HE (Shawcross et al. J Hepatol 2004;40:247-254; 2011;54:640-9). Another link between inflammation, ammonia and HE is through gut flora and endotoxins (Bajaj JS et al. Am J Physiol Gastrointest Liver Physiol. 2012;302:G168-75; Solga and Diehl. Hepatology 2004;39:1197-1200; Gupta et al, J Hepatol;53:849-855). Cirrhotic patients with MHE have substantial derangements in the gut microecology, with significant fecal overgrowth of potentially pathogenic *Escherichia coli* and *Staphylococcal* species (Liu *et al*. Hepatology 2004;39:1441–1449) and a decrease in the count of Bifidobacterium (Zhao et al. Chin. J. Dig.2004; 5: 64–7). Probiotics, through manipulation of intestinal flora, have emerged as a therapeutic option for MHE and OHE treatment without significant adverse effects. Prebiotics, probiotics or synbiotics might be efficacious in the treatment of HE by decreasing bacterial urease activity, pH in the gut lumen, ammonia absorption and total ammonia in the portal blood, and by improving nutritional status of gut epithelium resulting in decreasing intestinal permeability. In addition, they help ameliorate the inflammation and oxidative stress in the hepatocytes, leading to increased hepatic clearance of ammonia. Previous trials showed that probiotics are efficacious in treating HE. However a recent systematic review analysed 7 trials to quantify the beneficial and harmful effects of any probiotic in any dosage, compared with placebo or no intervention, or with any other treatment for patients with any grade of acute or chronic HE [McGee RG, et al. Cochrane Database Syst Rev. 2011 Nov 9;(11):CD008716]. When probiotics were compared with no treatment, there was no significant difference in all-cause mortality, lack of recovery, adverse events and quality of life. Plasma ammonia concentration was significantly lower for participants treated with probiotic at one month but not at two months. Plasma ammonia decreased the most in the participants treated with probiotic at three months. The trials suffered from a high risk of systematic errors ('bias') and high risk of random errors ('play of chance'). While probiotics appeared to reduce plasma ammonia concentration when compared with placebo or no intervention, probiotics were not efficacious in altering clinically relevant outcomes. We conducted 2 double blind, randomised, parallel groups, and placebo controlled trials to assess the beneficial effects of VSL#3® supplementation (900 billion viable lyophilised bacteria) (i) in the reduction in MHE prevalence in cirrhotic patients without any previous history of HE and (ii) on the prevention of recurrence of overt HE (secondary prevention). Secondary outcome included: improvement in health related quality of life (HRQOL), cognitive functions, liver and renal functions, prevention of major complications of cirrhosis, blood ammonia levels, blood cytokines levels, hospital admissions due to cirrhosis complications and survival. VSL#3 supplementation did not change the primary outcome while its supplementation was associated with improvement in serum IL-6 levels, Child-Pugh score, partial improvement in parameters of cognitive functions, fewer infective complications and fewer hospitalizations.
Conclusion: Demonstration of unequivocal efficacy is needed before probiotics can be endorsed as effective therapy for HE.

O38 ROLE OF ANTIBIOTICS

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The modification of gut flora has been the mainstay of HE therapy and recent evidence has shown dysbiosis in HE and cirrhotic patients. A variety of antibiotics have been studied for modifying this dysbiosis in MHE and OHE; most of which have been gut-selective and non-absorbable. Treatment trials for OHE have centered on improvement on mental status or reduction of recurrence after control of the episode. Antibiotics studied have included neomycin, paromomycin, metronidazole, vancomycin and rifaximin. Although neomycin is FDA-approved for HE, its use is limited by oto-and nephro-toxicity. Vancomycin and metronidazole use have also fallen out of favor because of resistance(VRE) and neurotoxicity respectively. Currently the strongest evidence base out of the antibiotics studied for OHE is for rifaximin. There are trials starting from the 1980s that have demonstrated its efficacy in OHE management, improvement in quality of life (QOL), reduction in recurrent HE episodes and cost of hospitalizations. The latest trial showed that in conjunction with lactulose, rifaximin was able to prevent recurrent HE and HE-related hospitalizations. On the other hand, the treatment of MHE using antibiotics is still evolving, particularly due to the short duration of current MHE trials and the specter of cost and resistance. Rifaximin has been shown to improve cognition, QOL and driving simulator performance but longer trials with endpoints such as prevention of OHE are needed. While it is assumed that antibiotics destroy the harmful bacteria, they could also modulate the bacteria to become less virulent without eliminating them. Risks for long-term use are unclear, specifically with regards to resistance, C.difficile and cost and systematic studies of the interactions between probiotics, antibiotics and non-absorbable disaccharides are still needed. Antibiotics, especially non-absorbable molecules, are a mainstay of therapy for OHE and are emerging as promising alternatives for MHE therapy.

O39 NONABSORBABLE DISACCHARIDES AND HEPATIC ENCEPHALOPATHY

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Hepatic encephalopathy (HE) is a challenging clinical complication of liver dysfunction with a wide spectrum of neuropsychiatric abnormalities that range from mild disturbances in cognitive function and consciousness to coma and death. The uncertainties on the pathogenesis of HE limit the development of specific pharmacological therapies but a key role is thought to be played by circulating gut-derived toxins of the nitrogenous compounds, most notably ammonia. Management of HE primarily involves avoidance of precipitating factors. The use of non absorbed disaccharides to reduce the colony of ammonia producing gut flora and to decrease the systemic absorption of ammonia from the intestinal lumen forms the mainstay mainstay of current guidelines for the management of HE. The nonabsorbable disaccharides include lactulose and lactitol. Nonabsorbable disaccharides are considered the first-line therapy for treatment of acute HE, and improvement in symptoms occurs in 67% to 87%.Lactitol is comparable to lactulose in the treatment of HE with fewer side effects. Lactulose has also shown to be effective in primary and secondary prophylaxis of HE. Lactulose significantly improves cognitive function and health-related quality of life in patients with minimal hepatic encephalopathy. In a systematic review, the efficacy of nonabsorbable disaccharides was compared with either no intervention or placebo; the overall treatment effect was modest but statistically significant, with a relative risk of no

improvement ranging from 0.62 to 0.92. There is insufficient evidence to support or refute the use of non-absorbable disaccharides for HE. Another recently published meta-analysis comparing rifaximin versus nonabsorbable disaccharides concluded that rifaximin is not superior to nonabsorbable disaccharides for acute or chronic hepatic encephalopathy in the long-term or short-term treatment except that it may be better tolerated.

O40 RIFAXIMIN FOR THE TREATMENT OF HEPATIC ENCEPHALOPATHY; A META-ANALYSIS OF RANDOMISED STUDIES

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Introduction: The non-absorbable antibiotic rifaximin has been used to treat hepatic encephalopathy (HE) since the 1980s. The aim of this study was to evaluate its efficacy and safety for the treatment of all types of HE in patients with cirrhosis against a variety of comparative regimens.
Methods: A language unrestricted search for papers published between January 1983 and January 2012 was undertaken utilizing 11 databases; randomized treatment trials were identified. Raw data were obtained for six trials. Data were extracted on (i) the numbers of patients showing improvement overall; (ii) the means (±1SD) for mental state, asterixis, number connection tests (NCT), EEG and blood ammonia; and (iii) adverse events. Fixed effect model meta-analyses were applied, by population type. Random effects analyses were used when results showed significant heterogeneity. Overall relative proportions were calculated for binary outcomes and standardized mean differences (SMD) for quantitative outcomes.
Results: Seventeen trials were identified comparing rifaximin to non-absorbable disaccharides, other antibiotics or 'no treatment' in patients with acute, chronic, or minimal HE or else for its prevention. Most trials were of adequate quality; the total sample size was 1037 patients, but data were not extractable for all outcomes. The relative proportion of patients experiencing clinical improvement with rifaximin, compared to all control regimen, was 1.26 (95% Confidence Interval [CI] 1.05 to 1.52, p=0.014). Broadly consistent benefits were shown for individual outcome variables e.g. EEG grade (SMD -0.49, 95% CI -0.92 to -0.07, p=0.023) and blood ammonia (SMD=-0.25, 95% CI -0.48 to -0.02, p=0.034). SMDs were stronger in the eight trials in chronic HE. No significant differences were observed in side-effects between rifaximin and control regimens.
Conclusion: Rifaximin is an efficacious and safe treatment for HE when compared to a variety of other treatments regimens; the benefits are most marked in patients with chronic HE.

O41 TOLL-LIKE RECEPTOR- 4 : A NOVEL TARGET FOR THERAPY OF HEPATIC ENCEPHALOPATHY

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(Y Sharifi, N Shah and D Adebayo contributed equally to this study)

Introduction: The pathogenesis of hepatic encephalopathy (HE) involves a synergistic interaction between ammonia and inflammation. The effectiveness of a non-absorbable antibiotic, Rifaximin in HE points to the important role of the gut – liver –brain axis. Toll like receptor 4 (TLR4), is a ligand for pathogen and damage associated molecular patterns, which when stimulated leads to inflammation and is expressed in a variety of

brain structures primarily microglia, astrocytes and neurons. We hypothesised that reduction on gut translocation or administration of a TLR 4 antagonist to a well-described model of HE would reduce brain edema.
Materials and Methods: Cirrhosis was induced in rats with bile-duct ligation (BDL). Administration of LPS (1mg/Kg) to these animals results in coma and increase in brain water. Study 1: 6 groups rats: [Sham, Sham+LPS, BDL,BDL+LPS, , BDL+Norfloxacin (20mg/kg administered orally daily for 10 days) and BDL+ Norfloxacin+LPS. All the animals were sacrificed at 3 hours. Time to coma was studied in a separate group. Study 2: A novel TLR4 anatgonist, IAXO (5mg/kg) (Innaxon ltd.) was given IP or SC Sham+vehicle, Sham+IAXO+LPS, BDL+LPS+vehicle, , BDL+IAXO+LPS. Biochemistry, plasma and brain cytokines, arterial ammonia and frontal cortex brain water were measured.
Results: Selective gut decontamination by Norfloxacin was associated with a significant reduction in plasma and brain cytokines, NFkB, brain water and delay in onset of coma in the BDL animals administered LPS with a reduction in the brain expression of TLR4 ($p<0.02$ resp). TLR4 antagonist (IAXO) administration resulted in significant reduction in cytokines and brain water in the BDL+LPS animals compared with BDL+LPS+controls without significant changes in ammonia.
Conclusion: The results this study describes TLR4 as a novel target of therapy in HE which is independent of ammonia and points to important role of the gut – liver – brain axis in the pathogenesis of HE.

SESSION 11: ELECTROLYTES, WATER CONTENT AND TRANSPORTERS

O42 UREA-CYCLE DEFECTS AND HYPERAMMONEMIA: EFFECTS ON FUNCTIONAL IMAGING

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Ornithine transcarbamylase deficiency (OTCD) is an X-linked urea cycle disorder characterized by hyperammonemia resulting in a number of neurological alterations. We have been using advanced neuroimaging to study a large cohort of patients through a Urea Cycle Rare Disorders Consortium supported by the NIH. Over the past 7 years we have used structural MRI, FLAIR imaging, diffusion tensor imaging, 1H MRS, and fMRI to study the underpinnings of neurological injury. We have found white matter injury manifest as decreased fractional anisotropy in frontal white mater, biochemical alterations (elevated glutamine and low myoinositol) and impairments in working memory and executive cognition on fMRI. The current presentation will focus on our use of fMRI to investigate differences in BOLD signal activation between subjects with OTCD and healthy controls during a working memory task. We studied 19 subjects with OTCD and 21 healthy controls. An N-back working memory task was performed in a block design using 3T functional magnetic resonance imaging. Results: In subjects with OTCD we observed increased BOLD signal in the right dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) relative to healthy age matched controls. We found increased neuronal activation in OTCD subjects despite equivalent task performance points to sub-optimal activation of the working memory network in these subjects, most likely reflecting damage caused by hyperammonemic events. These increases directly relate to our previous finding of reduced frontal white matter integrity in the superior extents of the corpus callosum; key hemispheric connections for these areas. Future studies using higher cognitive load are required to further characterize these effects.

O43 OXIDATIVE METABOLISM AND BRAIN OEDEMA

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Acute liver failure with persistent hyperammonemia is associated with a risk of intracranial hypertension due to brain oedema. Microdialysis studies have revealed that in the brain tissue of such patients there is a linear relationship between lactate to pyruvate (LP) ratio and glutamine as well as ATP degradation products. This is in concordance with in vitro studies of brain tissue and cell cultures that have found that exposure to ammonium 1) inhibits enzymatic activity in the tricarboxylic acid (TCA) cycle, 2) induces TCA cycle substrate depletion through marked glutamate utilization for glutamine synthesis and 3) leads to mitochondrial dysfunction. However, alternative interpretations of these clinical in vivo findings should be considered and will be discussed in a systematic presentation of the conflicting data within this topic.

O44 HYPONATRAEMIA, HEPATITIS C, AND DIABETES ARE RISK FACTORS FOR HEPATIC ENCEPHALOPATHY IN LARGE PROSPECTIVE STUDIES IN DECOMPENSATED CIRRHOSIS

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Hepatic encephalopathy (HE) is one of the most common manifestations of decompensated cirrhosis, but current knowledge of frequency, characteristics and predictive factors is mostly derived from small studies of patients with a previous history of HE.
Ambulant patients in three controlled trials of satavaptan in cirrhosis with ascites were followed for up to 1 year. Patients with HE>grade 1 at screening or with TIPS were excluded. Complications of cirrhosis were recorded every 4 weeks. Kaplan-Meier analysis was used for estimating 1-year event rates and a multivariate Cox model for relative risk (RR).
1198 patients were included: 69.9% male and 30.4% female, with a mean age 57.2 years, mean MELD score 13.3 and 26% in Child-Pugh class C. Most frequent etiologies were alcohol (68.4%), HCV (21.1%), HBV (8.4%). 139 patients (11.6%) were hyponatraemic (NaCl < 130 mmol/l) on entry and 306 patients (25.6%) had a history of HE. Median duration of observation was 240 days.
In total 395 episodes of HE were recorded. 264 patients (22.0%) experienced at least one spisode (1-year event rate 27.1% (95%CI 24.1, 29.9)) and 169 patients (14.1%) had an episode leading to hospitalization or death. The highest grade of HE was grade 4 in 28 patients (2.3%), grade 3 in 44 patients (3.7%) and grade 2 in 94% patients (7.9%). Precipitating factors reported (n=239) included infections (24.7%), renal insufficiency (9.2%), dehydration (8.4) and variceal bleeding (7.5%). Episodes of HE were associated with history of HE (RR 2.22), hyponatraemia (RR 2.01), serum bilirubin > 51µmol/l (RR 2.11), diabetes (RR 1.40) and hepatitis C aetiology of cirrhosis (RR 1.65).
This large study found that infections are the most common precipitating factors of HE. In addition to previous history of HE and poor liver function, this study identified hypenatraemia, hepatitis C infection, and diabetes as risk factors for HE.

O45 INCREASED EXPRESSION OF AQUAPORIN-4 IN PERIVASCULAR ASTROCYTES END-FEET CONTRIBUTE TO THE DEVELOPMENT OF BRAIN EDEMA IN ACUTE LIVER FAILURE

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Background and Aims: Intracranial pressure caused by cerebral edema and associated astrocyte swelling remain major cerebral complication of acute liver failure (ALF), the mechanisms responsible for which have yet to be identified. In vitro and in vivo studies on ALF reveals significant increase in expression of AQP-4, a water channel protein contributes to the astrocyte swelling leading to brain edema and intracranial pressure in ALF (Neuroreport 2003;14:2379-82; J Neuropathol Exp Neurol 2010;69:869-879; Metab Brain Dis 2010; 25:315-323). However, there is no evidence available to date to suggest that these alterations occur in patients with ALF. Methods: In order to address this issue, dissected samples of cerebral cortex were obtained at autopsy from 8 patients with ALF

due to either viral hepatitis or toxic liver injury (mean age 26 years; range 13-46) and from 7 control patients with no evidence of liver or other neurological disorders (mean age 54 years; range 16-78). All ALF patients had high grade hepatic encephalopathy and, there was evidence of brain edema in all ALF patients on neuropathology. The expression of the AQP-4 at mRNA level was evaluated by using real time PCR and protein expression was assessed using both immunoblotting (western) techniques as well as immunohistochemistry using commercially-available polyclonal antibodies. Results: AQP-4 mRNA expression was significantly up-regulated by 3.38 folds (P=0.003) but its protein level in total cell lysate from frontal cortex of ALF patients remained unchanged (P=0.3) compared to controls. However, immunhistochemical analysis showed increase in AQP-4 immunoreactivity in the plasma membrane around the perivascular astrocyte end-feet and forming a continuous perivascular sheath in both grey and white matter in ALF patients compared to controls. Conclusion: These findings suggest that over expression of AQP-4 plasma membrane levels in perivascular astrocyte end-feet are likely to contribute in the development of brain edema in ALF.
Acknowledgement: The study was financially supported by Indian Council of Medical Research (ICMR) (ID: 53/18/2008-BMS).

SESSION 12: THE BRAIN IN LIVER FAILURE

O46 LIVER FAILURE AND THE BRAIN: A LOOK THROUGH THE CRYSTAL BALL

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Hepatic encephalopathy (HE) is a complex neuropsychiatric syndrome which is associated with liver dysfunction and remains a major clinical problem in patients with cirrhosis. Furthermore, HE is an important component of diagnostic component in acute liver failure when 25% of patients with acute and hyperacute aetiologies may develop brain oedema and intracranial hypertension resulting from astrocyte swelling. In cirrhosis, encephalopathy is more insidious causing a range of neuropsychiatric abnormalities which include psychomotor retardation, impaired memory, increased reaction time, sensory abnormalities and poor concentration. When HE is severe in cirrhosis, patients may develop varying degrees of confusion and coma.

The evidence base supporting a significant role for ammonia is robust, but in clinical practice a consistent correlation between the concentration of ammonia in the blood and the manifest symptoms of HE is not always seen. Potential synergy between ammonia and inflammation has been observed, and there is a compounding effect of hyponatraemia. These interactions are likely to be further developed, understood and manipulated to control brain failure in patients with liver disease and sepsis in future years.

Management of the cirrhotic with HE should concentrate upon treating potential precipitants (infection, dehydration and variceal bleeding). Clinical manifestations are those of confusion and loss of day : night cycle and progression to decreased Glasgow coma scale with the potential to result in micro-aspiration and further sepsis. Management may require protecting the airway with intubation and ventilation. Future point of care testing is likely to see earlier diagnosis of altered neurocognitive function in the community and wards by application of novel biomarkers probably detected through placing a patient on a smart phone screen and analysis of the secretions from the skin. Depending on the profile detected with knowledge of the patients genetic predisposition and manipulations and interventions will be applied in a personalized format.

Treatment of HE per se is mainly focused upon use of ammonia lowering agents, such as lactulose. In addition there is a potential role for other ammonia lowering agents such as LOLA. More recently there has been further interest in the use of non-absorbable antibiotics (neomycin and metronidazole) following a positive publication using Rifaxamin. In the future drug therapies will be clinically available with focused effects central and systemic inflammation will be available to us in addition to drugs which will modulate endothelial function in various vascular beds including the blood brain barrier.

In acute liver failure there are risks of rapidly changing level of HE and the risk of cerebral oedema with intracranial hypertension. Patients with acute liver failure and encephalopathy should always be referred to specialist units, since encephalopathy may, along with other criteria, require listing for transplantation. The development of highly effective agents to minimize or even prevent encephalopathy in patients with acute liver failure may limit greatly the accuracy of present prognostic models for transplantation. New models will need to be developed to account for a situation where a patient may have gross hepatic necrosis and lack the potential for effective regeneration but not demonstrate encephalopathy.

O47 DOES LIVER ASSIST HELP PATIENTS WITH HEPATIC ENCEPHALOPATHY?

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ALF is a clinical syndrome with multiorgan failure. The management aims to support vital organ function while waiting for the liver to recover by regeneration. However, an efficient liver assist device is needed especially in the most severe and rapidly deteriorating cases to gain time until liver transplantation can be performed.
The metabolic dysfunction and the pathophysiological mechanisms that result in hepatic encephalopathy (HE) and multiorgan dysfunction are complex and poorly understood in ALF. One hypothesis suggests that intoxication is responsible, while the other point of view holds that deterioration of the clinical condition is related to lack of factors produced by the normal liver (the "fall-out concept"). Apart from the accumulation of waste metabolites and toxic substances, the "fall-out concept" also takes the vital synthetic, regulatory, and metabolic liver functions into consideration.
Plasma exchange (PE) corrects coagulopathy in ALF patients, and also removes hepatotoxins, large proteins released from the failing liver as well as pro-inflammatory cytokines. Most studies performed with membrane cell separators have exchanged a volume corresponding to the plasma volume. High-volume PE is based on the concept of exchange of a plasma volume equally to the total extracellular space of the patient. This has later been redefined (for logistic reasons) to exchange of a plasma volume of 10 L per day for 3 days.
The hyperdynamic systemic circulation in patients with ALF is characterised by low arterial pressure, and a low systemic vascular resistance. PE increases mean arterial pressure by 30 % and systemic vascular resistance by 60 %. Systemic oxygen delivery decreases by 25 % (as CO decreases), whereas oxygen consumption remains constant as the oxygen extraction ratio increases by ~20%. Cerebral blood flow increases by ~40 % and cerebral oxygen metabolism ($CMRO_2$) by ~55 %. Also studies in patients with ALF shows that hepatic urea synthesis is accelerated, and more recently a RCT on PE strongly indicated that it improves survival.
Single or repeated treatment with other “detoxification systems” such as albumin dialysis have shown very similar effects on blood pressure as well on vital organ blood flow and metabolism. One RCT in ALF patients, awaiting emergency transplantation did not show any effect of MARS on survival compared to a control group but the current concept of supporting these ALF patients is to do PE in the early stage of HE stage 3-4 (i..e. ASAP after intubation) by 1-2 treatment of a volume of about 10 litres (replaced 1:1 by FFP) and then follow by intermittent or continuous albumin dialysis together with (high-volume) CvvHD. However, a new RCT is needed to support this concept of extracorporeal treatment and is currently planned and will focus on non-transplant candidates.

O48 NEUROINFLAMMATION IN THE PATHOGENESIS OF THE CNS COMPLICATIONS OF ACUTE LIVER FAILURE

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It is well established that systemic inflammation worsens encephalopathy and brain edema and its complications in acute liver failure (ALF) and more recently, evidence for the presence of neuroinflammation (inflammatory processes in the brain per se) has been accumulating. Evidence in favor of neuroinflammatory mechanisms includes the finding of microglial activation in brain (microglia are the immunomodulator cells of the brain) together with increased brain accumulation of proinflammatory cytokines such as TNF-a, IL-1b, and IL-6. Although the precise nature of the signaling mechanisms

between the failing liver and the brain leading to neuroinflammation is unknown, mechanisms involving blood–brain cytokine transfer and brain lactate have been proposed. Neuroinflammatory responses in liver failure result in upregulation of translocator protein (TLP), a mitochondrial membrane protein that is particularly concentrated in microglia. Positron emission tomography studies using the TLP ligand C-PK11195 in cirrhotic patients with mild HE reveal increased signals consistent with microglial activation and neuroinflammation. It has been proposed that existing therapies for HE including lactulose, rifaximin, albumen dialysis, and probiotics have the potential to lower both circulating ammonia and
proinflammatory cytokines. Moreover, mild hypothermia and N-acetylcysteine have similar joint actions. Treatment of experimental animals with liver failure due to liver ischemia or toxic liver injury reveals that minocycline, an agent with potent inhibitory actions on microglial activation or the TNF-a receptor antagonist etanercept, leads to slowing of progression of encephalopathy and brain edema in ALF. Translation of these findings to the clinic has the potential to provide novel strategies for the management and treatment of the CNS complications of liver failure in the future.

O49 HEPATIC ENCEPHALOPATHY RECEDES AFTER LIVER TRANSPLANTATION BUT COGNITIVE FUNCTION DOES NOT NORMALIZE

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Introduction: Liver transplantation (LTX) is the final therapeutic option for patients with end-stage liver disease, of whom at least one third develop hepatic encephalopathy (HE). The impact of LTX on cognitive function in patients with and without preceding hepatic encephalopathy (HE) is not well established. This study aims to follow-up cognitive function before and after LTX.
Methods: Sixty-one patients awaiting liver transplantation underwent psychometric testing with Portosystemic Encephalopathy -Syndrom-Test (PHES), Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), Inhibitory Control Test (ICT) and Critical Flicker Frequency (CFF) measurement before LTX and were followed-up for 6 months after LTX. 36 completed 12 months follow-up. Patients were subdivided into those with (n=10) and without hepatic encephalopathy (n=51) before LTX according to their clinical presentation, PHES or ICT Target score.
Results: Before LTX the HE group did significantly worse ($p< 0.01$) than the non-HE group with regard to the mean PHES, CFF, and RBANS total score, but not the ICT scores. 6 months follow-up the two groups differed significantly with regard to PHES and RBANS. Psychometric data are available from 43 patients, since 11 patients died and 7 refused or missed follow-up. Of interest patients without cognitive dysfunction before LTX did worse in RBANS 6 months after LTX than before ($p=0.033$) and did not improve until their 12 months-follow-up. Patients with cognitive dysfunction showed significantly improved PHES ($p=0.038$) and ICT Targets ($p=0.011$) after LTX while CFF and RBANS showed only a positive trend and ICT lures no change.
Discussion: Patients with hepatic encephalopathy prior to LTX show an improvement of cognitive function after transplantation. The cognitive deterioration in patients who were unimpaired before LTX, however, hints to a possible –perhaps transient - negative impact of the LTX surgery on cognitive function or alternatively to a possible negative impact of immunosuppression.

O50 ROLE OF OXIDATIVE STRESS IN PREVENTION OF BRAIN EDEMA DURING AN ACUTE DETERIORATION OF CHRONIC LIVER FAILURE

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Aims: Acute-on-chronic liver failure (ACLF) represents an acute decompensation of liver cirrhosis. End-to-side portacaval anastomosis (PCA) followed by hepatic artery ligation (HAL) performed after 4 weeks represents a model of liver decompensation. In this model, the onset of coma is delayed compared to acute liver failure induced by hepatic devascularisation. As oxidative stress plays a role in brain edema in chronic liver failure, the objective of this study was to investigate the role of oxidative stress in the pathogenesis of brain edema in ACLF.
Methods: Male Sprague-Dawley rats were subjected to PCA followed by hepatic artery ligation (HAL) either concomitantly (HAL-0) or 4 weeks (HAL-4W) following shunt surgery or to a SHAM intervention. Body temperature and blood glucose were monitored and maintained throughout the experiments. Brain edema (specific gravimetric technique) and glutathione levels (spectrophotometry) were measured in brain tissue of all groups.
Results: Brain water content was significantly attenuated in “acute-on-chronic” rats (SHAM: 80.12±0.09 %; HAL-0: 81.39±0.15 % ($p<0.01$ vs SHAM); HAL-4W: 80.04±0.13 % (ns vs SHAM; $p<0.01$ vs HAL-0)). Arterial ammonia concentration followed a similar pattern (control: 0.060±0.007 mM; HAL-0: 1.340±0.090 mM ($p<0.001$ vs SHAM); HAL-4W: 0.350±0.070 mM ($p<0.001$ vs SHAM; $p<0.001$ vs HAL-0)). Glutathione levels did not change in HAL-4W compared to SHAM and were significantly decreased in the brains of HAL-0 rats (by 36% vs SHAM, $p<0.05$ and by 25% vs HAL-4W). These effects were not due to an improvement in liver function, as liver necrosis markers AST and ALT did not differ between HAL-4W and HAL-0 rats.
Conclusions: Brain edema, ammonia levels and oxidative stress are reduced in ACLF rats compared to acute liver failure rats. These findings suggest that during chronic liver failure compensatory mechanisms are developed that prevent the apparition of brain edema and attenuate oxidative stress during an acute deterioration.

SESSION 13: INFLAMMATIN AND HEPATIC ENCEPHALOPATHY

O51 NEUROINFLAMMATION IN COGNITIVE IMPAIRMENT AND HYPOKINESIA IN HYPERAMMONEMIA AND HEPATIC ENCEPHALOPATHY.

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We have shown that rats with portacaval-shunts (PCS), bile duct ligation (BDL) or chronic hyperammonemia without liver disease show neuroinflammation, with microglial activation and increased inflammatory markers (IL-6, iNOS, COX activitiy, PGE-2) in certain brain regions.

Chronic moderate hyperammonemia without liver failure is enough to induce microglial activation and neuroinflammation.

Treatment of PCS, BDL or hyperammonemic rats with an anti-inflammatory, ibuprofen, reduces microglial activation and neuroinflammation, restores the function of the glutamate-NO-cGMP pathway in cerebellum in vivo and learning ability of rats. Ibuprofen also eliminates hypokinesia in PCS rats, restoring motor activity.

Ibuprofen and other NSAIDs may induce kidney damage in cirrhotic patients. We looked for a treatment that could reduce microglial activation and neuroinflammation in brain without inducing secondary effects in kidney. We found that inhibition of p38 reduces microglial activation and neuroinflammation in PCS rats and restores learning ability without affecting kidney function.

These data support that neuroinflammation plays a main role in the cognitive and motor alterations in chronic hyperammonemia and HE and that reducing neurinflammation improves the neurological status in animal models.

We are also studying the contribution of hyperammonemia and inflammation to the neurological alterations in patients with minimal HE (MHE). IL-6 and IL-18 levels are higher (2.5-fold) in serum from patients with MHE than in those without MHE. There are significant correlations between IL-6 or IL-18 levels and PHES score and CFF. This supports that inflammation plays a relevant role in cognitive impairment in MHE.

We also assessed mild cognitive impairment in patients with different types of liver or dermatological diseases resulting in different grades of hyperammonemia and/or inflammation. Hyperammonemia and inflammation act synergistically and the combination of certain levels of hyperammonemia and inflammation is enough to induce cognitive impairment, even in the absence of liver disease.

O52 SYSTEMIC INFLAMMATION AND AMMONIA

Debbie L. Shawcross

Infection and inflammation have been associated with the development of delirium for many centuries and there is a rapidly growing evidence base supporting the role of inflammation in exacerbating the neurological manifestations of both acute and chronic liver failure. Inflammation can be categorised into that which arises directly within the brain itself resulting in astrocytic, microglial and neuronal dysfunction, impacting on the development of encephalopathy and ‘brain failure’. Inflammation may also develop systemically and indirectly influence brain function and this will be the focus of this talk. I will describe some of the mechanisms that underpin the development of systemic inflammation in liver failure including the role of hyperammonemia and the ‘cytotoxic soup’ of pro-inflammatory mediators which are released into the peripheral circulation from the necrotic liver in acute liver injury. I will also discuss how systemic inflammation has been shown to influence the cerebral impact of arterial ammonia.

Systemic inflammation and ammonia induce neutrophils to degranulate ('toxic degranulation') and release reactive oxygen species into the peripheral circulation where they may ultimately cross the blood brain barrier. Circulating endotoxin which arises from the gut (bacterial translocation) and superimposed sepsis, in addition to ammonia exposure, in patients with liver failure also influence the expression of microbial pattern recognition receptors such as Toll-like receptors in the innate immune system. Neutrophil toll-like receptors 4 and 9 have recently been shown to one of several receptors upregulated in response to the development of hyperammonemia in acute liver failure and variceal bleeding in patients with cirrhosis and encephalopathy and may offer one of several novel therapeutic targets.

O53 ENCEPHALOPATHY AND LIVER TRANSPLANT

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Liver transplant candidates experience frequently episodic or persistent hepatic encephalopathy. In addition, these patients can exhibit neurological comorbidities, such as small vessel cerebrovascular disease, that contribute to cognitive impairment in the pre-transplant period. Assessment of the respective contribution of hepatic encephalopathy or comorbidities in the cognitive manifestations is critical to estimate the neurological benefits of restoring liver function. Magnetic resonance imaging and spectroscopy are useful to assess the impact of liver failure or comorbidities. This assessment is critical to decide liver transplant in difficult cases.

In the early postoperative period, liver transplant is commonly complicated by a confusional syndrome. The possible role persisting hepatic encephalopathy in its development has not been clearly established. The origin is usually considered multifactorial and relates to disturbances present before transplant, such as hepatic encephalopathy and complications following liver transplant, such as infections, rejection, primary liver dysfunction, immunosuppressors, etc… The diagnosis and treatment is based in the recognition of comorbidities and optimal care of metabolic disturbances. Several psychoactive drugs can be use to control neurological manifestations.

Several studies have demonstrated recovery of cognitive function after liver transplant in patients with minimal hepatic encephalopathy. However, some deficits may persist specifically among patients with persistent HE. Those patients with high glutamine have highest potential of improving cognitive function after liver transplant. Other factors present before liver transplant that contribute to a worse neuropsychological outcome after liver transplant are diabetes mellitus and alcohol consumption. Long term after liver transplant, cognitive function may worsen in relation to vascular risk factors. Motor dysfunction is usually less severe, but even severe cases of mielopathy may improve following liver transplant.

O54 BACTERIAL INFECTIONS INCREASE THE INCIDENCE OF BOTH OVERT AND MINIMAL HEPATIC ENCEPHALOPATHY: RESULTS OF A PROSPECTIVE STUDY

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Bacterial infections in cirrhotic patients are well known precipitating factors for overt hepatic encephalopathy (OHE) while the relationship with minimal HE (MHE) is less clear. Neurocognitive disorders, known as "sepsis-associated encephalopathy" (SAE) have also been reported during severe infections also in non-cirrhotic patients. Our study was aimed at investigating the relationship between neurocognitive disorders and infection/inflammation in cirrhotic vs non-cirrhotic patients.
We enrolled in the study consecutive cirrhotic patients referred at our University Hospital. Patients with known neurological disease were excluded. Patients were divided into 3 groups: sepsis, infections without sepsis, no infections. Hospitalized patients without liver or neurological diseases, matched for age and sex, were studied as control group. A pool of standardized questions, evaluating the time and space orientation, was used for the diagnosis of OHE or SAE. Three paper and pencil tests (TMT-A, TMT-B and DST) were use for the diagnosis of MHE. One-hundred-fifty cirrhotic patients (age 64±13 years; Child B-C 70%) and 81 controls were included. Sepsis was diagnosed in 31 cirrhotics and 23 controls and infections without sepsis in 19 cirrhotics and 10 controls. Neurocognitive disorders were recorded in 90% of cirrhotics with sepsis(29% OHE and 61% MHE), in79% of cirrhotics with infections (42% OHE and 37% MHE), only in 42% of non infected ones (17% OHE and 25% MHE) ($p \leq 0.01$). In the control group, none had a diagnosis of SAE, subclinical neurocognitive alterations were detected only in patients with sepsis (42%). The total rate of neurocognitive disorders was always higher in the cirrhotic group ($p \leq 0.01$). Infections seem to be a trigger not only for OHE but also for MHE. Although subclinical neurocognitive alterations may arise also in the general population during sepsis, their prevalence is higher in cirrhotic patients.

O55 SYSTEMIC OXIDATIVE STRESS INDUCTION LEADS TO BRAIN EDEMA IN HYPERAMMONEMIC PORTOCAVAL-SHUNTED RATS

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Aims: Although ammonia is the central component in the pathogenesis of hepatic encephalopathy, it is believed oxidative stress (OS) plays a role in the pathogenesis of brain edema during liver disease. We previously demonstrated that portocaval shunted hyperammoniemic rats do not develop OS or brain edema (Bosoi et al., FRBM, 2012). To define whether a synergistic effect exists between hyperammonemia and OS in the development of brain edema, the present study investigates the role of OS in the pathogensises of brain edema in PCA rats following glutathione depletion by diethyl maleate (DEM).
Methods: We evaluated the effect of DEM (1 mg/kg/day intraperitoneally for 10 days starting day 18 after surgery) in PCS and SHAM-operated rats. Rats were sacrificed at day 28 and OS was evaluated by measuring arterial and cerebrail malon-dialdehyde (MDA, commercial kit), reactive oxygen species (ROS, DCFDA fluorescence), and 4-hydroxy-2-nonenal (HNE, Western blot). Ammonia levels (commercial kit) were also assessed. Frontal cortex brain water was measured using a specific gravimetric technique.
Results: DEM induced a significant increase in plasma MDA, ROS and HNE levels in PCA rats. No increase was detected in DEM-treated SHAM-operated controls. No

changes in cerebral OS markers were observed in any group. Ammonia levels increased in non-treated and DEM-treated PCA vs SHAM-operated rats ($p<0.001$) and remained unchanged between non-treated and DEM-treated PCA groups. Brain water content increased in DEM-treated PCA rats vs non-treated PCA rats (PCA+DEM; 78.45±0.13% vs PCA: 77.38±0.11%, $p<0.001$).
Conclusions: DEM treatment in PCA rats induced systemic, not central OS. This, superimposed on hyperammonemia, led to the development of brain edema. Similar DEM doses did not results in increased OS and brain edema in SHAM-operated rats, suggesting PCA rats are more susceptible to OS. Our findings support a synergistic effect between hyperammonemia and systemic OS in the pathogenesis of brain edema in hepatic encephalopathy.

P1 IN VIVO IMAGING OF THE CEREBRAL TRANSLOCATOR PROTEIN-BINDING CAPACITY IN PATIENTS WITH LIVER CIRRHOSIS

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Aim: The astrocytic mitochondrial translocator protein (TSPO) is suggested playing a key role in the pathogenesis of hepatic encephalopathy (HE). Two studies investigated TSPO in the brain of patients with liver cirrhosis and HE in vivo by analyzing the binding of the TSPO-radioligand C-11-PK11195 via positron emission tomography (PET). The first study showed a significantly increased C-11-PK11195-binding especially in the basal ganglia in a group of patients with minimal HE and cirrhosis of different etiology including alcohol abuse and hepatitis C virus (HCV) infection. This finding could not be confirmed by the second study which included exclusively patients with overt HE due to alcoholic cirrhosis. Recently an increased C-11-PK11195-binding in the caudate nucleus and thalamus was shown in patients with mild chronic hepatitis C. This study aims to clarify if the cerebral C-11-PK11195-binding in patients with HCV cirrhosis exceeds that of patients with mild chronic hepatitis C.

Methods: 5 patients with HCV cirrhosis without HE, 6 healthy controls and 14 patients with mild hepatitis C underwent neurological examination, psychometric testing and C-11-PK11195-PET/CT. C-11-PK11195-binding was assessed based on voxelwise application of a reference tissue model. After spatial normalisation of 3D data sets mean binding was extracted for the frontal, temporal and occipital cortex, thalamus, pallidum, putamen, caudate nucleus, amygdala, cerebellum and pons.

Results: PET showed a significantly increased C-11-PK11195-binding capacity in the three cortical regions, the pallidum, putamen and caudate nucleus ($p<0.05$) in the cirrhotic patients compared to both other groups. The distribution of TSPO-binding differed, too: in patients with cirrhosis C-11-PK11195-binding peaked in the pallidum and putamen, in controls and non-cirrhotic HCV patients in the thalamus.

Conclusion: Increased C-11-PK11195-binding in patients with HCV cirrhosis is a consequence of cirrhosis not of HCV infection. Further studies should address the impact of different grades of HE and other etiologies of liver disease.

P2 ETANERCEPT DELAYS PROGRESSION OF ENCEPHALOPATHY IN ACUTE LIVER FAILURE BY BOTH HEPATIC AND CENTRAL ANTI-INFLAMMATORY MECHANISMS

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Encephalopathy and brain edema are serious CNS complications of acute liver failure (ALF). The mechanisms responsible have not been fully elucidated but previous reports suggest that pro-inflammatory cytokines (Chastre et al., Hepatology 52(4) (suppl.1):1086A, 2010) and oxidative/nitrosative stress (Bémeur et al., Metab Brain Dis., 25(2):241-9, 2010) play a role. To further address these issues, the effects of etanercept (10 mg/kg; ip.), a TNF-α neutralizing molecule, were investigated in mice with ALF resulting from azoxymethane hepatotoxicity. The progression of encephalopathy was assessed as a function of inflammatory and oxidative/nitrosative stress markers in liver

and brain. Temperature and glycemia were rigorously controlled. In both liver and brain, GSH/GSSG ratios were measured by colorimetric assay, nitrites/nitrates by the Griess method, alanine aminotransferase (ALT), aspartate aminotranferase (AST) by automated analyser, and IL-6 was measured by ELISA. Etanercept treatment delayed the progression of encephalopathy and onset of coma (vehicle vs. etanercept : 20.4h ± 0.34h vs. 27.8h ± 0.95h; $p < 0.001$), normalized plasma transaminases and significantly reduced the IL-6 levels in both plasma and brain ($p< 0.05$). Etanercept increased GSH/GSSG ratios in both brain (vehicle vs. etanercept : 319.9 ± 43.23 vs. 507.4 ± 61.21; $p < 0.05$) and liver (204.1 ± 24.28 vs. 418.0 ± 39.58; $p < 0.05$) and concomitantly decreased nitrite/nitrate levels ($p< 0.05$). These results (i) confirm a key role of systemic and central neuroinflammatory mechanisms in the pathogenesis of encephalopathy in ALF, (ii) demonstrate a significant therapeutic benefit of etanercept and (iii) suggest that the drug's beneficial effects are mediated by both peripheral (improvement of hepatic function) and central anti-inflammatory mechanisms.

P3 ORAL ZINC SUPPLEMENTATION FOR HEPATIC ENCEPHALOPATHY: SYSTEMATIC REVIEW AND META-ANALYSIS

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Background. Has been postulated low serum zinc levels as precipitating for hepatic encephalopathy. Therefore zinc supplementation is considered to be a therapeutic option. Objectives. To assess the effects of oral zinc supplementation in the treatment of hepatic encephalopathy. Methods. Data sources; Electronic databases (The Cochrane Library, MEDLINE, EMBASE) and handsearch (the references of all identified studies). Study eligibility criteria; Prospective randomized clinical trials. Participants and interventions; Adult patients diagnosed with liver cirrhosis and hepatic encephalopathy. Types of interventions; Any oral zinc supplementation versus no intervention, placebo, or other interventions for management of hepatic encephalopathy. Study appraisal and synthesis methods; Data was analyzed calculating the relative risk (RR) for each trial, expressing the uncertainty with 95% confidence intervals (CI). Continuous data were analyzed calculating standard mean differences (SMD) between groups of each trial and its 95%CI. Statistical heterogeneity was defined as a P-value > 0.10 ($\chi 2$) or I2 > 25%. Results. Were included four trials (233 patients). A significant improvement in number connection test was observed with oral zinc supplementation (SMD -0.54; 95%CI -0.90 to -0.19), without reduction in the rate of encephalopathy recurrence (RR 0.64; 95%CI 0.26 to 1.59). Limitations. There is heterogeneity in the outcomes reported in the included trials. Precluding evidence based analysis of significant outcomes on hepatic encephalopathy. Conclusions. Oral zinc supplementation improve number connection test. However there is no evidence-based information regarding other clinical or biochemical outcomes.

P4 METABOLIC PROFILING USING PROTON NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF PLASMA IN PATIENTS WITH CIRRHOSIS CORRELATES WITH ARTERIAL AMMONIA BUT NOT GRADE OF HEPATIC ENCEPHALOPATHY

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Introduction: Diagnosing overt hepatic encephalopathy (HE) in patients with cirrhosis relies on subjective clinical examination. Arterial ammonia correlates poorly with differing grades and a useful blood test for diagnosis and monitoring is lacking. Metabolic profiling by nuclear magnetic resonance (NMR) spectroscopy may offer diagnostic biomarkers in overt HE.
Methods: Thirty-seven patients with cirrhosis and differing grades of HE were identified. Arterial blood was drawn for arterial ammonia, biochemical analysis and NMR spectroscopy. HE was diagnosed and graded by West-Haven criteria. ^{1}H NMR spectroscopy was performed in a Bruker 600 MHz Avance spectrometer using a Carr-Purcell-Meiboom-Gill sequence and multivariate analysis performed using orthogonal partial least squares discriminant analysis (OPLS-DA).
Results: Twenty-four male and 13 female patients, 21 with alcohol-related cirrhosis, 5 viral, 11 mixed/other causes, with median (range) age 57(35-75) years made up the study cohort. Fifteen patients had no clinically detectable HE, 15 had grades 1-2 and 7 grades 3-4. Median Model for End Stage Liver Disease (MELD) score was 14 (4-32) and median arterial ammonia level 106 (19-268 µmol/L). Ammonia level correlated weakly with HE grade (Kendall's tau=0.238, p=0.040) but not with MELD score (tau=0.010, p=0.118). No multivariate models using HE grade as a categorical (OPLSDA) or continuous (OPLS) variables resulted in validity (OPLS model: R2(Y) = 0.489, Q2(Y) = -0.091). A 2-component OPLS model using ammonia as a Y variable identified multiple metabolites closely correlating with arterial ammonia (R2Y=0.566, Q2Y=0.394, cross-validated ANOVA p=0.0008). Metabolites associated with high ammonia levels included pyruvate, 3-hydroxybutyrate, glutamate, phenylalanine and an unassigned resonance at 2.43 ppm. Resonances associated with high-density lipoproteins correlated negatively with ammonia.
Conclusion: Plasma ^{1}H NMR spectroscopy does not discriminate between grades of HE in patients with cirrhosis and overt HE. Multiple metabolic pathways correlate with arterial ammonia level. Alternative metabolic profiling techniques such as mass spectroscopy may be required to assist in diagnosis in these patients.

P5 METABOLIC MAPPING OF ORNITHINE IN CIRRHOTIC RATS TREATED WITH ORNITHINE PHENYLACETATE

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Hyperammonemia is considered to be the main causative factor of hepatic encephalopathy. Recently, a novel concept for ammonia removal has been proposed consisting of the combined administration of ornithine and phenylacetate (OP), which has shown ammonia lowering effects in rat, pig and human studies. However, the metabolic pathway of ornithine during the treatment with OP is not elucidated. Ornithine is thought to increase formation of glutamine by the skeletal muscle, and glutamine is removed by the conjugation with phenylacetate forming phenylacetylglutamine (PAGN). Final disposal of nitrogen is thought to occur by excretion of PAGN into the urine. In the present study we investigated the interorgan metabolism of ornithine and ammonia in OP treated cirrhotic rats. Our results show that the majority of ornithine derived glutamine in arterial blood is released from the liver and a minor part originates from skeletal muscle. OP treatment only leads to a transient ammonia lowering effect together with a momentary increase in arterial glutamine levels. The metabolite PAGN could not be detected in urine or blood samples in OP treated cirrhotic rats. This

transient effect of OP can very well be explained by the lack of PAGN formation in the rat. When glutamine is not conjugated to phenylacetate the ornithine-derived-glutamine will instead be deamidated into glutamate and ammonia by glutaminase present in the kidney and gut, thus reversing the beneficial ammonia lowering effect of increased glutamine synthesis.

P6 ALTERED CIRCUMVENTRICULAR SECRETION IN CIRRHOTIC RATS: POSSIBLE INVOLVEMENT OF 5-HT DEPLETION

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Whether brain structures with strategic positions in the interface of blood-brain barriers such as the circumventricular organs are involved in hepatic encephalopathy (HE) is not yet established. Among the circumventricular organs, the subcommissural organ (SCO) secretes a glycoprotein known as Reissner's fiber (RF), which condenses and forms an ever-growing thread-like structure into the cerebrospinal fluid.
We describe RF material within the SCO and its serotonergic (5-HT) innervation in an animal model of chronic HE following bile duct ligation in rats. The study involved immunohistochemical techniques with antibodies against RF and 5-HT.
Four weeks after bile duct ligation surgery, a significant rise of RF immunoreactive material was observed in all SCO areas compared with controls. Moreover, significant RF-immunoreactive materials within the ependyma and inside the parenchyma close to the ventricular borders were also seen in bile duct ligated rats, but not in control rats, suggesting that the overproduction of RF material contribute possibly to liberation of RF within other brain structures such as CSF, and periventricular area . In BDL rats, we observe a significant reduction of 5-HT immunolabelling of the SCO, the ventricular borders and the nucleus of origin, the Dorsal Raphe Nucleus (DRN). Based on the literature it is evident that RF material in rats is under control of 5-HT system, suggesting that reduction of 5-HT in BDL rats may driven FR accumulation within the SCO and the ependymal borders.
Our data describe alterations of the SCO/RF material probably due to a general 5-HT deficit in BDL rats. We hypothesise that neurotoxins accumulated in HE may contribute to a 5-HT system deficit in DRN and subsequent projections to the SCO and the ependymal area with consequence on SCO/RF materials aggregation in CSF and brain parenchyma.

P7 3-NITRO-TYROSINE AS A PERIPHERAL BIOMARKER OF MINIMAL HEPATIC ENCEPHALOPATHY IN PATIENTS WITH LIVER CIRRHOSIS

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Patients with liver cirrhosis may present minimal hepatic encephalopathy (MHE) that can be unveiled using specific neuropsychologic examination. Early detection of MHE would be very useful. The "gold standard" for MHE diagnosis is the psychometric hepatic encephalopathy score (PHES). It would be very useful to have some blood biomarker

reflecting the presence of MHE in cirrhotic patients. The aim of this work was to identify serum molecules useful as biomarkers for MHE.
We measured in 63 controls, 43 cirrhotic patients without MHE, and 44 patients with MHE, serum levels of different amino acids, cyclic guanosine monophosphate (cGMP), and 3-nitrotyrosine. We analyzed for each parameter its diagnostic accuracy as an indicator of MHE, as assessed using the PHES.
These studies supported that 3-nitro-tyrosine is a good marker for MHE. To validate its utility as a biomarker for MHE, we analyzed in a second cohort of 44 cirrhotic patients without MHE and 18 patients with MHE, serum levels of 3-nitro-tyrosine, methionine, and citrulline. Citrulline (173 ± 17%), methionine (173 ± 16%), and 3-nitro-tyrosine (857 ± 92%) were increased in sera from patients with MHE when compared with those without MHE. The receiver operating characteristic (ROC) curve analysis of 3-nitro-tyrosine for the diagnosis of MHE in the first cohort showed an area under the curve (AUC) value of 0.96 (95% confidence interval 0.93 – 0.99). At the cutoff of 14 nM, the specificity was 93%, sensitivity 89%, and positive and negative predictive values were both 91%. When the same cutoff was applied to the second cohort, the specificity was 83% and sensitivity was 94%. The positive and negative predictive values were 70 and 97%, respectively.
Conclusions. This pilot study shows that determination of 3-nitro-tyrosine in serum is useful to identify patients with MHE, with good sensitivity, specificity, and positive and negative predictive values.

P8 EFFECTS OF REDUCTION OF LIVER MASS PRIOR TO AZOXYMETHANE-INDUCED ACUTE LIVER FAILURE IN MICE

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Current murine models of acute liver failure (ALF) are not ideal for studying the cerebral complications of ALF, as most pharmacological and genetic manipulations may directly affect the extent of liver damage. As a potential solution, we investigated the effects of performing a partial hepatectomy (PH) prior to the induction of ALF in mice treated with azoxymethane.
We performed a PH in C57Bl6 mice (7-12 week-old) followed 16 h later by the administration of azoxymethane (62.5 or 100 ug/g bw i.p.). Non-operated mice treated with azoxymethane or saline were also studied. Body temperature and glycemia were monitored, and we measured brain water content (dry/wet brain weight ratio), plasma ammonia, ALT, bilirubin, and plasma IL-6.
The administration of azoxymethane induced severe liver damage in both non-operated and PH mice, as assessed both histologically and by the elevation of plasma ALT. The administration of azoxymethane in mice with PH resulted in increased susceptibility to hypoglycemia and hypothermia, in higher hyperammonemia (50.4 +/- 11.1 vs. 144.5 +/- 55.6 uM at 8 h, $p< 0.05$), and in attenuated elevations of plasma ALT and bilirubin compared with non-operated mice treated with azoxymethane. The time to coma was significantly shorter in non-operated mice compared with mice with PH (17.33 +/- 0.42 h vs. 20.94 +/- 1.65 h, $p< 0.05$). Both groups of azoxymethane-treated mice appeared to have similar systemic inflammatory response as assessed by the levels of plasma IL-6. We were not able to detect differences in brain water content between any of the groups.
Our results suggest that factors other than ammonia may influence the development of the cerebral complications in ALF. The development of slight hypothermia and/or the

attenuated release of necrotic products from the liver could be potential explanations for the increased time to coma after azoxymethane administration in mice with PH.

P9 CAN TRANSIENT ELASTOGRAPHY BE USED TO PREDICT THE PRESENCE OF MINIMAL HEPATIC ENCEPHALOPATHY IN A COHORT OF COMPENSATED CIRRHOTIC PATIENTS?

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Introduction: Minimal hepatic encephalopathy (mHE) is a common cause of neurocognitive dysfunction in patients with cirrhosis. It is associated with falls, impaired driving skills, the later development of overt HE and reduced overall survival (1-4). Treatment has been shown to improve psychometric performance and enhance quality-of-life parameters (5). The diagnosis is based on psychometric and/or neuro-physiological tests but a consensus on a gold standard test has yet to be reached. These tests are time consuming and currently not widely used outside of the research setting. Transient elastography (TE) is an established non-invasive tool to determine the severity of hepatic fibrosis.
Aim: The aim of this study was to investigate if TE could be used in a population with compensated cirrhosis to identify patients most likely to have mHE.
Methods: All compensated cirrhotic patients attending the outpatient department over a six-month period were included in the study. We included patients with a TE score of >16kPa or a biopsy showing cirrhosis. Each patient completed the Psychometric Hepatic Encephalopathy Score (PHES) and had TE performed on the same day. PHES raw data was compared to UK normative data (unpublished) and a score of two or more standard deviations below the mean was considered consistent with a diagnosis of mHE.
Results: 14/50 patients (28%) had mHE on PHES. TE score was significantly higher in those with mHE than in those without mHE (Median 45.5 v 17.5; p=0.001). Of the 14 patients with a TE score >32kPa, 10 patients (71%) had mHE. Of the 36 patients with a TE score <32kPa, 4 patients (11%) had mHE. The sensitivity and specificity of using a LSM of 32kPa or greater to predict the presence of mHE were 71% and 89% respectively. Conclusion: TE can be used to risk stratify patients for the presence of mHE. All patients with a TE score of >32kPa should either be tested for mHE or empirically treated.

Sincere thanks to Dr Marsha Morgan for facilitating the use of UK PHES normative data
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P10 EFFECT OF ALBUMIN INFUSION ON THE DEVELOPMENT OF HEPATIC ENCEPHALOPATHY (HE) AFTER THERAPEUTIC PARACENTESIS. A SYSTEMATIC REVIEW

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Background: The pathogenesis of HE remains poorly understood. Circulatory disturbances, inflammation and oxidative/nitrosative stress have been implicated in its pathophysiology. Albumin, a multifunctional protein, is the main intravascular antioxidant and acts as endotoxin scavenger, thereby reducing inflammation. Its concentration and function are known to be decreased in liver failure. Albumin has been largely used as a plasma expander after large volume paracentesis and the aim of this study is to determine whether this prevents the development of HE.
Objective: To assess whether albumin infusion can prevent/minimize HE development following paracentesis.
Methods: We performed a systematic review of randomized trials evaluating albumin infusion in patients with tense ascites following paracentetis. Eligible trials were sought by computer database search. Non randomized trials, including shunts, ascites reinfusion, TIPS or albumin in the control group were excluded. To test the association between albumin treatment and HE, an exact Fisher test and odd ratios with confidence interval at 95% were calculated for each study.
Results: Nine studies with 774 patients were included. Four studies had a small size (<30 patients/group). No study reported a definition for HE or a diagnostic protocol. Six of them reported an adequate randomization method. The follow-up period was heterogeneous (5-224) days. Treatment strategies in control groups included no therapy (n=2), diuretics (n=1), colloids (n=6). None of the studies showed a significant benefit of albumin in preventing the development of HE: p-values for Fisher test was greater than 0,3 an confidence intervals for odd ratios included number 1 (Figure 1).
Conclusions: This systematic review of studies, which though not addressed to diagnose and quantify the development of HE, does not show a benefit of albumin infusion in preventing HE following paracentetis. Further studies which include precise diagnosis protocol and short-term follow-up are needed to address this question.

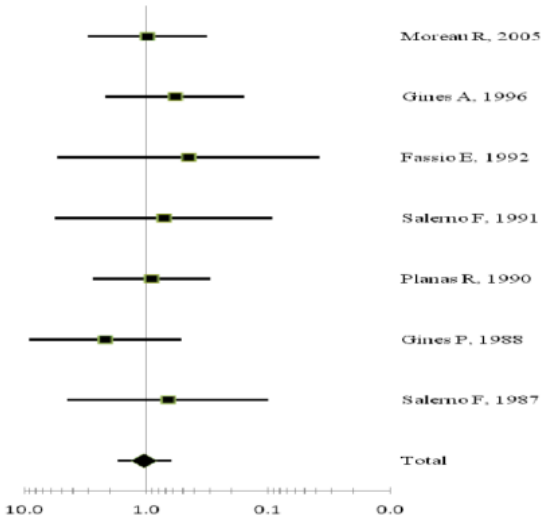

Figure1: Odd ratios and confidence interval at 95%

P11 ALTERED NRF2 GENE EXPRESSION LEADS TO EXIDATIVE STRESS IN ACUTE HYPERAMMONEMIC RATS

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Background and aim: Studies on models of acute hyperammonemia (AHA) suggested a role of oxidative stress in neuropathology of ammonia toxicity. Nrf2 [Nuclear factor (erythroid derived 2)-like 2] is a transcription factor which regulates expression of antioxidant genes at transcription level and protects a cell against oxidative stress. Nrf2 expression is found to be altered in oxidative stress conditions in neurological diseases [J Neuropathol Exp Neurol 2007;66:75-85). In order to address this issue, we investigated Nrf2 gene expression along with oxidative stress parameters in frontal cortex and cerebellum of AHA rats. Material and methods: AHA was induced in Wistar rats (180-200g) by intra-peritoneal injection of ammonium acetate (10x103 and 8x103 µmol/kg b.w.) at half an hour interval. Control rats were given saline with respect to AHA group. Blood ammonia levels in blood and oxidative stress parameters in frontal cortex and cerebellum were investigated spechtrophotometrically, where as reactive oxygen species (ROS) in blood were measured by flow cytometric method. Nrf2 gene expression in frontal cortex and cerebellum was investigated by real time PCR. Results: Blood ammonia levels were increased ($P<0.001$) in AHA rats when compared to control rats. Nrf2 expression was reduced both in frontal cortex and cerebellum regions ($P<0.001$). Blood ROS levels were higher ($P<0.01$). Lipid peroxidation levels increased both in frontal cortex ($P<0.05$) and cerebellum ($P<0.01$). Superoxide dismutase activity decreased both in frontal cortex ($P<0.05$) and cerebellum ($P<0.01$). Catalase activity decreased only in cerebellum ($P<0.01$). Activity of glutathione peroxidase ($P<0.05$) and glutathione reductase ($P<0.05$) and glutathione levels ($P<0.05$) were decreased in only frontal cortex. Conclusion: Nrf2 gene expression decreases significantly in AHA rats. Decreased Nrf2 gene expression may be one of the factors in ammonia-induced oxidative stress in AHA.

P12 THE PERFORMANCE VALIDITY OF BREATH SAMPLE ANALYSIS IN THE DIAGNOSIS OF HEPATIC ENCEPHALOPATHY IN PATIENTS WITH CIRRHOSIS

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Introduction: The purpose of this study was to evaluate breath sample analysis for volatile organic compounds (VOCs) in the diagnosis of hepatic encephalopathy (HE) in patients with cirrhosis.

Methods: The patient population comprised 26 individuals (17 men, 9 women) of mean (range) age 60 (45-75) years, with biopsy-proven cirrhosis secondary to alcohol (n=21), or non-alcoholic steatosis, hepatitis C, autoimmune hepatitis, haemochromatosis or cryptogenic (n=1 each). Patients were classified using clinical, psychometric and electroencephalographic variables as neuropsychiatrically unimpaired (n=10) or as having minimal (n=6) or overt (n=10) HE. Breath samples were collected in a chemically inert 3L Tedlar bag (Adtech, UK). Two litres of gas were evacuated into an empty glass tube which was packed with graphitised carbon adsorbents able to trap a wide range of VOCs (Sigma Aldrich, UK). Each tube was processed using a TurboMatrix automated thermal desorption unit (PerkinElmer, UK) attached to a Clarus 500 gas chromatograph mass spectrometer. In total 280 discrete peaks from the chromatographic output were investigated for potential use as markers of HE.

Results: A number of peaks were identified in the patients with cirrhosis which were absent or present in significantly different quantities in ten healthy controls. Discriminant analysis allowed the generation of two classification equations using data from 12 peaks to build a predictive model for HE. This model correctly classified all patients from the original population (Figure).
Conclusion: Analysis of VOCs identifies patients with HE with a high degree of accuracy. Future work will validate the classification equations in a new group of patients and identification of the individual compounds involved will provide insights into the pathogenesis of the syndrome and potential new therapeutic targets.

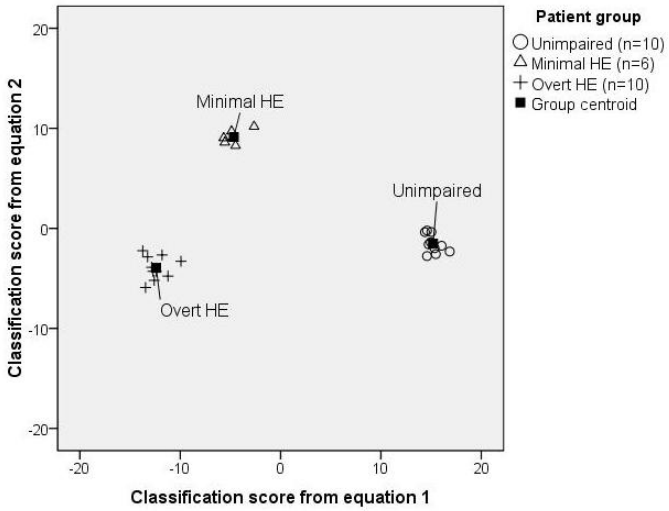

Figure: Classification scores for the model used to predict HE.

P13 THE DIAGNOSIS OF HEPATIC ENCEPHALOPATHY: TEST PERFORMANCE OF NOVEL PARADIGMS

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Introduction: The current validated methods for diagnosing hepatic encephalopathy (HE), the psychometric hepatic encephalopathy score (PHES) and analysis of the electroencephalogram (EEG), are not universally available. Recent interest has focused on novel diagnostic tools: the Sternberg paradigm, critical flicker fusion (CFF) frequency, smooth pursuit eye movements (SPEM) and the Inhibitory Control Test (ICT).
Aim: To assess the performance of these tools in the diagnosis of HE in patients with cirrhosis.
Methods: The patient population comprised 51 individuals (33 men, 18 women) of mean (range) age 54 (36–74) years, with biopsy-proven cirrhosis. Patients were classified using clinical, psychometric and electroencephalographic variables as either neuropsychiatrically unimpaired (n=14) or as having minimal (n=24) or overt (n=13) HE. The control population comprised 111 healthy volunteers (52 men, 59 women) of mean age 39 (21–70) years. The Sternberg paradigm, CFF, SPEM and ICT were evaluated using standardised procedures.
Results: The Sternberg paradigm had the best performance characteristics: a threshold of 1706.4ms on the 4-set negative probe reaction time diagnosed any degree of HE with a sensitivity of 80.0% and a specificity of 68.8%. The other tests performed less well. The best test combination was the Sternberg paradigm and SPEM, although the contribution of SPEM did not reach statistical significance. A model built using these two tests diagnosed any degree of HE with a sensitivity of 67.9% and a specificity of 74.2%.
Conclusion: The proposed diagnostics are not particularly easy to perform; some are particularly difficult to administer to patients at the severe end of the disease spectrum. None of the tests have excellent performance profiles and they do not fulfil the brief of a

single test to replace the gold standard assessment consisting of mental state examination, PHES and EEG.

P14 CONTINUOUS REACTION TIME (CRT) MESUREMENTS, A METHOD STUDY

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Background: Whereas the diagnosis of hepatic encephalopathy is a clinical one once other causes of confusion has been excluded, the diagnosis of minimal hepatic encephalopathy requires neuropsychological testing. A psychometric test battery is gold standard, but cumbersome, and less suitable because of a learning effect when applied repeatedly. Instead, neurophysiological tests such as critical flicker frequency, inhibitory control test or continuous reaction time measurement have been suggested as objective and sensitive tests. However, the clinical significance of abnormal tests in patients without ouvert encephalopathy is unclear and there is no consensus on which test to use. Furthermore evaluations of the various tests are scanty. Aim: To determine the reproducibility and accuracy of continuous reaction time measurements. Methods: 32 patients with cirrhosis (6 with ouvert encephalopathy), 15 normal persons and 5 inpatients without liver disease, encephalopathy and use of sedatives were each tested 8 times in the course of 2 days, 4 tests per day with 75 minutes interval. 150 measurements of reaction time were performed per test. Results(CRT-index) were expressed as median reaction time divided by the 90-10 percentile. Patients with cirrhosis were rated clinically and liver function assessed by calculation of MELD and Child-Pugh scores before testing. One way ANOVA, wilcoxon matched-paires signed-ranks test and Mann- Whitney paired and un-paired t-tests were applied when appropiate. Results: The Index (mean +/- SEM) was 1,570 +/- 0,276 in cirrhotics with ouvert encephalopathy, 2,215 +/- 0,122 in cirrhotics without ouvert encephalopathy, 2,804 +/- 0,172 in healthy controls and 2,051 +/- 0,377 in inpatient controls. Intraperson variation was significantly lower than interperson variation, in cirrhotics with ouvert encephalopathy (0,112 versus 3,296), in cirrhotics without ouvert encephalopathy (0,123 versus 3,087) and in healthy controls (0,181 versus 3,570). A significantly lower reproducibility was found when index was based on 100 measurements instead of 150 measurements. A learning effect could not be demonstrated as no significant difference was found neither when first and last measurements, nor when results of day 1 and day 2 were compared . Positive and negative predictive values in detecting ouvert encephalopathy were 0.33 and 0.94. Conclusion: Continuous reaction time measurements seem biological meaningful, but with insufficient precision and not useful for the detection of encephalopathy in patients with cirrhosis of the liver. The possible influence of inpatient status should be further delineated. Further studies should be performed in patients with and without minimal hepatic encephalopathy and results compared to results of recommended psychometric tests

P15 MUSCLE GLUTAMINE SYNTHETASE PLAYS A CRITICAL ROLE IN AMMONIA HOMEOSTASIS IN ACUTE LIVER FAILURE

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INTRODUCTION & AIM: In patients with disease urea synthesis is diminished. It is hypothesised that the muscle glutamine synthetase (GS) plays an important role in

ammonia homeostasis but direct proof for this hypothesis is lacking. This study was performed in GS knockout mice (GS-KO/M) to test the hypothesis that muscle can act as an alternate site for ammonia detoxification.
METHODS: GS-KO/M mice were obtained by a selective, but complete elimination of GS expression in striated muscle of MCK-Cretg/-/GSfl/fl (GS-KO/M) mice. 4 groups of animals were studied: FVB flox mice expressing normal GS levels (WTf) and GS-KO/M received paracetamol (acetaminophen) (IP 250 mg/kg) to induce liver failure (ALF), or saline (IP) (n=5 in each group). We measured plasma levels for: ammonia and standard biochemical markers (AST, ALT, bilirubin, urea, lactate, glucose, creatinine) by COBAS (Roche Diagnostics). Brain water was measured by the dry weight method and ammonia was measured in tissue homogenates. GS protein expression was determined by western blotting in liver and in leg muscle.
RESULTS: Plasma ammonia was found elevated in WTf ALF vs. WTf (122.00 ± 37.29 vs. 58.87 ± 9.87 μmol/L) and further increased in GS-KO/M ALF after 8 hours (299.5 ± 7.50 μmol/L). Brain ammonia was increased in GS-KO/M ALF vs WTf ALF mice (53.98 + 5.10vs.45.79+2.65 nmol/mg wet tissue). Brain water was increased in GS-KO/M ALF vs. WTf ALF (79.82 ± 0.47 vs. 78.72 ± 0.42%; $P<0.05$)- Plasma biochemistry measures were not different between the paracetamol treated groups, indicating a consistent level of liver injury.
CONCLUSION: The results of this study provide direct evidence for first time of muscle GS acting to metabolize systemic ammonia in liver failure, with associated reduction in brain water. This suggests a possible role for strategies of augmentation of GS expression as a therapy for liver dysfunction.
ACKNOWLEDGMENT: Prof. WH Lamers and Prof. TB Hakvoort for providing the GS-KO/M models, Amsterdam (Netherlands).

P16 AMMONIA IMPAIRS BDNF SIGNALLING IN CULTURED RAT ASTROCYTES

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Introduction: Ammonia induced glutamine accumulation in astrocytes plays a key role in pathogenesis of hepatic encephalopathy (HE) [1]. Intracellular signaling of brain-derived neurotrophic factor (BDNF) through its receptor tyrosine receptor kinase B (TrkB) strongly enhances neurotransmission an dynamically shapes astrocyte morphology which is essential for maintenance of synaptic architecture[2]. Disturbed BDNF signaling is associated with impaired neurotransmission and memory formation. However, the impact of ammonia on BDNF mediated TrkB-signalling in astrocytes is currently unknown.
Aims: The present investigation was conducted to characterize effects of ammonia on BDNF and TrkB mRNA and protein expression level and to analyse its impact on BDNF-mediated morphology changes in cultured rat astrocytes.
Results: NH4CL time-and concentration-dependently decreases TrkB mRNA expression level and concurrently inhibits N-glycosylation of TrkB protein. Defective TrkB N-glycosylation is prevented by blocking glutamine synthetase using methionine sulfoximine (MSO) but cannot be mimicked by hypoosmotically (205mosmol/l)-induced astrocyte swelling. Whereas fully N-glycosylated TrkB is only detected in membrane preparations of untreated astrocytes, unglycosylated TrkB is found in both, cystosolic as well as in membrane preparations of NH4CL (5mmol/l) treated astrocytes. However, unglycosylated TrkB failed to transduce BDNF signalling towards actin polymerization, which underlies morphological changes in astrocytes.

Similar to TrkB N-glycosylation inhibition, ammonia time-and concentration-dependently increased O-linked N-acetyl-D-glucosaminidation of proteins (O-GlcNAc) due to increased utilization of glutamine within the hexosamin pathway. Raising O-GlcNAc levels either by increasing glutamine synthesis through NH4CL (5mmol/l) treatment or inhibition of O-GlcNAc removal using the GlcNAcase inhibitor PUGNAc both impaired TrkB N-glycosylation.
Discussion: The study suggests that ammonia disrupts BDNF-induced TrkB activation through an O-GlcNAc mediated inhibition of TrkB N-glycosylation and impairs BDNF mediated astrocytic morphology changes necessary for stabilisation of synaptic contacts. This provides a new mechanism by which ammonia via glutamine might contribute to impaired neurotransmission in hepatic encephalopathy.
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P17 DIAGNOSTIC VALUE OF CFF AND PHES IN MINIMAL HEPATIC ENCEPHALOPATHY

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Background and Aims: Critical Flicker Frequency (CFF) as well as the Psychometric Hepatic Encephalopathy Score (PHES) test are widely used to diagnose Minimal Hepatic Encephalopathy (MHE). These test systems were compared with respect to their diagnostic value for MHE using computerpsychometric tests and adapted West-Haven-criteria as reference.
Methods: A total of 820 patients (261 controls and 559 cirrhotics) was enrolled and tested by computerpsychometry and determination of CFF; out of these 148 patients were evaluated by a complete (5 paper and pencil tests) PHES tests for specificity and sensitivity in detecting MHE. 448 patients were investigated by an incomplete, debugged (4 paper and pencil tests) PHES test for the same reasons.
Results: Whereas CFF on the basis of the 39 Hz borderline separated well between HE0 and overt HE for the whole study population (N=820; sensitivity 98%; specificity 93%) as well as for the 148 patients (sensitivity 97%; specificity 100%), separation by the complete PHES test in the same patient population on the basis of the established borderline -4 was lower (84 %). The 39 Hz borderline did separate MHE from HE0 only with a sensitivity of 40% (N=820) resp. 24% (N=148) as did the complete PHES test (sensitivity:32%, specificity:84%). Interestingly, sensitivity (49%) and specificity (73%) of incomplete PHES with a borderline of -1, assessed after use of leave-one-out-validation, was even better for differentiation between HE0 and MHE than complete PHES test. Though the sensitivity is increased by combining linking CFF and incomplete PHES test to 62% in separating HE0 from MHE more than one-third of the patients are diagnosed still wrongly.
Conclusion: This diagnostic dilemma for MHE-grading can only be overcome by a fundamental change in the approach to HE-grading. Severity-grading should rely on an objective physical and reproducible parameter as CFF or incomplete PHES test which can quantitatively describe low-grade HE as a continuum from entire normality to unequivocal pathology.

P18 VALUE OF EXTERNAL AND SELF-ASSESSMENT SCALES FOR DIAGNOSTIC OF MINIMAL HEPATIC ENCEPHALOPATHY

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Background and Aims: Apart from objective test systems also Health related Quality of Life (HRQoL) scales are used to diagnose all aspects of human well-being, physical and cognitive skills, social functioning, and psychological status in patients with Minimal Hepatic Encephalopathy (MHE). Their usefulness in comparison to objective tests of MHE was never proven.
Methods: Apart from standard tests as computerpsychometry external assessment scales as Gottfries-Brane-Steen-scale (GBS) were compared with self-assessment-scales as the list-of-adjectives (EWL60S) and Munich-life-quality-dimension-list (MLDL) in Grading of MHE and HE.
Results: A total of 56 patients with liver cirrhosis were enrolled and graded by computerpsychometry, West-Haven-criteria (WHC) and CFF. 16% (N=9) were graded as HE0, 34% (N=19) as MHE, 34% as HE1 and 16% as HE2. Self-assessment scales (EWL60S, MLDL) showed in all parameters no relevance for severity grading of HE. Only the external assessment scale (GBS) showed differences between the global score for the different HE groups. In a analysis it was shown that there is a small correlation between CFF and GBS scale (r= -0,34; p=0,014), which showed that both parameters analyse different qualities. With a borderline of 39 Hz for separation between MHE and HE 0 the sensitivity (47 %) and specificity (89 %) is low. A combination of CFF (cut-off ≤ 39 Hz) and GBS scale (Cut-off: ≥ 8 points) showed that a point score of at least 1 increased the sensitivity for MHE diagnosis to 87.5% (Specificity: 54.5%).
Conclusion: In the diagnostics of MHE self-assessment scales are not helpful. CFF and GBS-scale alone have shown a low accuracy for separation of MHE to HE0 and HE1. By a combination of the GBS-score and CFF high sensitivities could be reached for diagnosing MHE. However a separation will not be possible.

P19 RIFAXIMIN IS A HIGHLY EFFICACIOUS TREATMENT FOR THE PARKINSONIAN PHENOTYPE OF HEPATIC ENCEPHALOPATHY (HE)

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Introduction: Patients who develop Parkinsonian symptoms on a background of cirrhosis and portosystemic shunting (PSS) form a unique subset of so-called acquired hepatocerebral degeneration. The syndrome differs from acute HE and idiopathic Parkinsonism, rarely responding to standard treatments for HE. Rifaximin is a non-absorbable antibiotic recently shown to be efficacious in secondary prevention of recurrent HE.
Methods: 3 patients referred to our HE clinic with cirrhosis, PSS and debilitating HE with parkinsonian symptoms were given rifaximin 600mg twice daily and prospectively followed. Their symptoms included resting tremor, bradykinesia, hypomimia and excessive somnolence. Each patient was independently evaluated by a hepatologist and a neurologist. Neuropsychological function testing (Trails A and B test), random venous ammonia (NH3), EEG and MRI brain/DaTscan were performed pre- and 4 weeks post-rifaximin.
Results: Patient 1 [male, age 61, α1AT, NH3 76 μmol/L] was unable to complete Trails A/B at baseline. On rifaximin his drooling and severe bradykinetic-rigid symptoms resolved and repeat Trails B test was in the 75th-90th centile for a normal age-matched

population. Patient 2 [female, age 64, alcohol, abstinent, NH3 67 µmol/L] experienced a dramatic reduction in somnolence and bradykinesia on rifaximin, with an improvement of Trails A from 10th to 50th centile. Patient 3 [male, age 66, alcohol, abstinent, NH3 67 µmol/L] demonstrated remarkable improvement in facial expression and mobility on rifaximin, being able to walk with assistance whereas previously he had required hoisting. None of the patients had improvement in ammonia levels or changes in EEG or MRI/DaTscan findings post-rifaximin despite obvious clinical improvement. Patient 1 has now been transplanted and his extrapyramidal and neurocognitive symptoms have resolved suggesting that the syndrome is reversible.
Conclusion: Rifaximin is efficacious in the treatment of the Parkinsonian phenotype of HE in this small case series, independent of ammonia lowering. Larger clinical trials are now warranted.

P20 OXIDATIVE STRESS MEDIATES RAPID ASTROCYTE SWELLING IN RESPONSE TO PRECIPITATING FACTORS OF HEPATIC ENCEPHALOPATHY

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Introduction: Findings derived from cell culture, animal models for hepatic encephalopathy (HE) as well as data from patients with liver cirrhosis an HE show an elementary role for an interplay between osmotic and oxidative/nitrosative stress in the pathogenesis of HE [1-3]. In cultured astrocytes HE-precipitating factors rapidly increase the production of reactive nitrogen and oxygen species (RNOS) within seconds or minutes [2]. However, no study so far investigated their short-term effects on astrocytic volume and volume of other brain cells.
Aims: The present study characterizes the impact of ammonia, proinflammatory cytokines and the benzodiazepines clonazepam/diazepam on rapid volume changes in cultured astrocytes using life-cell microscopy and 3-dimensional volume-reconstruction of cultured rat astrocytes. Comparatively, the impact of ammonia on microglial and neuronal cell volume was studied.
Results:NH4CL(0.5-5mmol/l), the proinflammatory cytokines tumor-necrosis factor-alpha (10ng/ml=, Interferon-gamma(100U/ml) and Interleukin-1beta (10ng/ml) as well as diazepam, but not clonazepam (both 10µmol/l) rapidly elevated astrocyte volume up to 130% of untreated controls within an observation period of 20min. Scavenging RNOS using epigallocatechin-gallate (1µmil/l) completely abolished NH4CL (5mmol/l), TNF-alpha (10ng/ml) or diazepam(10ng/ml) induced astrocyte swelling. Astrocyte swelling in response to NH4CL (5mmol/l) treatment was also inhibited by about 50% by inhibition of nitric-oxide synthases (LNMMA, 1mmol/l), NADHP-oxidase (apocynine, 3mmol/l), NMDA-receptors (MK801, 100 µmol/l), cyclooxygenases (indomethacin/ diclofenac, both 10µmol) or chelation of intracellular calcium (BAPTA-AM), 10µmol/l). Conversely, H202 (100µmol/l), PGE2(25-100ng/m) as well as glutamate (100µmol/l) or Ionomycin (1µmol/l) rapidly induced astrocyte swelling within 20min. As opposed to astrocytes, neither cultured microglia nor neurons exhibited volume change in response to NH4CL (5mmol/l) treatment.
Discussion: The present study shows that HE-precipitating factors rapidly induce astrocyte swelling within minutes through the induction of oxidative/nitrosative stress. Since molecular mechanisms underlying increased RNOS production by ammonia also apply to the induction of astrocyte swelling, this study substantiates a mutual interrelation between osmotic and oxidative stress in the pathogenesis of hepatic encephalopathy.

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and hepatic encephalopathy. Gastroenterology. 1994; 107: 1475-8. [2]Häussinger D, Schliss F. Pathogenetic mechanisms of hepatic encephalopathy. Gut. 2008; 57: 1156-65. [3]Görg B. Qvartskhava N, Bidmon HJ, Palomero-Gallagher N, Kircheis G, Zilles K, Häussinger D. Oxidative stress markers in the brain of patients with cirrhosis and hepatic encephalopathy. Hepatology. 2010;52:256-65

P21 CICLOSPORIN DOES NOT ATTENUATE INTRACRANIAL HYPERTENSION IN A RAT MODEL OF ACUTE HYPERAMMONEMIA

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Cerebral edema and intracranial hypertension are potentially fatal complications of acute liver failure. Ammonia is considered to play a crucial role by induction of impaired mitochondrial function and astrocyte swelling. The pathophysiology involves mitochondrial permeability transition and upregulation of the water channel aquaporin-4. Studies of cultured astrocytes have demonstrated that ciclosporin – an inhibitor of the mitochondrial permeability transition – attenuates ammonia-mediated astrocyte swelling. In this study we evaluated the effect of ciclosporin on intracranial pressure, cerebral water content, and aquaporin-4 in a rat model with portacaval anastomosis and acute hyperammonemia. We hypothesized that ciclosporin would attenuate the intracranial pressure, reduce cerebral water content and the expression of aquaporin-4.
24 male Wistar rats with a surgically constructed portacaval anastomosis were randomized to one of four groups receiving ciclosporin/vehicle and ammonia/saline infusion. Ciclosporin/vehicle was given intrathecally prior to the ammonia/saline infusion. The ammonia/saline infusion was given intravenously for four hours, while intracranial pressure and mean arterial pressure were recorded. At the end of the experiment the rats were decapitated and cortical and cerebellar brain tissue were analysed for water and aquaporin-4 content.
We found that ammonia infusion had a significant effect on ICP and on cortical and cerebellar water content, which all were higher in the groups receiving ammonia (two-way analysis of variance). Treatment with ciclosporin resulted in relevant cortical tissue concentrations of ciclosporin (> 0.2 micromolar) but did not lead to a reduction in intracranial pressure. Furthermore, ciclosporin did not attenuate the increase in cerebral water content, and did not affect aquaporin-4 expression. Conclusion: Our results demonstrate that intrathecally administered ciclosporin does not attenuate intracranial hypertension or brain edema in rats with portacaval anastomosis and hyperammonemia.

P22 GENDER AND AGE EFFECTS ON THE CONTINUOUS REACTION TIMES METHOD IN NORMAL PERSONS AND PATIENTS WITH CIRRHOSIS

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Background: Minimal hepatic encephalopathy (MHE) is a metabolic disorder of the brain that frequently occurs in patients with liver cirrhosis. MHE can lessen a patient's quality of life, but the condition is treatable when identified. The continuous reaction times (CRT) method is used in screening for MHE. However, gender and age effects on the CRT method are unknown and may confound the results. Aim: To standardise the CRT method outcomes for age and gender effects. Methods: We studied 92 adult volunteers and 181 patients with cirrhosis. All participants underwent a CRT evaluation. During the test, the motor reaction time to an auditory signal was measured 100 times. The 10th,

50th, and 90th reaction time percentiles of each test were recorded, and the CRT index was calculated as the 50th percentile/(90th percentile-10th percentile), as a measure of the individual stability of the reaction times. Results: In volunteers, men reacted faster than women and reaction times slowed with age. Neither gender nor age effects were present in the CRT index results. Patients with cirrhosis reacted slower than did volunteers. As in the case with the volunteers, male cirrhosis patients reacted faster than their female counterparts, and the young patients reacted faster than did the old. The patients' reaction times were characterised by instability, but as with the normal volunteers, gender and age had no effects on the CRT index for cirrhotic patients. Conclusion: Age and gender have effects on reaction time percentiles but not on the CRT index. Screening of patients with cirrhosis using the CRT index identifies brain dysfunction but is not subject to the effects of gender and age.

P23 ALBUMIN REPLACEMENT AND ENDOTOXIN REMOVAL USING A NOVEL LIVER SUPPORT DEVICE (UCL-ARSENEL) REDUCES ICP AND IMPROVES SURVIVAL BY MODIFYING INFLAMMATORY MARKERS AND NOT AMMONIA

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Background. Liver failure causes irreversible albumin damage, with an associated bacterial endotoxin load that induces inflammation. Ammonia an inflammation are synergistic in the pathogenesis of hepatic encephalopathy. It is not clear whether they are independent targets for therapy or whether a change in hyperammonia reduces inflammation. Am albumin replacement system with a novel endotoxin ligation (ARSeNEL) component was developed for use in liver failure, which does not change ammonia levels.

Methods: We tested the device in an acetaminophen model of acute liver failure (ALF). (16 pigs-8ALF, 5 ALF + UCL-ARSeNEL). Irreversible ALF was induced by accetaminophen via a jejunel catheter, confirmed by deranged clotting function (PT<30% normal). Treatment was UCL-ARSeNEL or CVVH control within 2hrs of ALF confirmation. The ARSeNEL device consists of three components; plasmapheresis, endotoxin and high cut-off (100 kDa) filters; with fresh frozen plasma replacing ultrafiltered plasma. Endpoints were; survival; ICP; heamodynamic parameters, standard biochemistry, cytokines; albumin damage, and plasma endotoxin levels.

Results. UCL-ARSe-NEL significantly increased survival post ALF (ALF 15.8 $\pm$ 2.4hrs vs UCL-ARSeNEL 23.8 $\pm$ 1.9hrs; p=0.02). ICP index (1.7 $\pm$ 0.07 vs 1.4 $\pm$ 1.58) was reduced in the device treated group. No significant changes in ammonia were observed, but the inflammatory markers such as cytokines IL8, IL6, IL1b, TNFa and neutrophil activation were all reduced in the ARSeNEL treated group compared with ALF control. Endotoxin was reduced by a quarter (1.99 $\pm$ o.18 Eu/ml vs 1.42 $\pm$ 0.21 Eu/ml) at 16hrs, noradrenaline requirement (61.11 $\pm$ 15.4 vs 28.7 $\pm$ 15.2 μg/Kg), and mean arterial pressure (71 $\pm$ 7.6 vs 87 $\pm$ 6.0 mmHg) showed marked improvement in the UCL-ARSeNEL group.

Conclusions. These results confirm that UCL-ARSeNEL reduces ICP and improves survival in ALF by modulating inflammation, through the ammonia levels not reduced. These data suggest that in liver failure, inflammation is an independent target of therapy.

P24 PLASMA PHOSPHOLIPID PROFILING BY METABONOMICS: HIGHLY ACCURATE OUTCOME PREDICTOR IN DECOMPENSATED CIRRHOSIS BUT A POOR PREDICTOR OF SEVERITY OF ENCEPHALOPATHY

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Introduction: Decompensated cirrhosis is associated with a high mortality and difficulty in outcome prediction. Phospholipids are altered in patients with liver cirrhosis and liver injury; reflecting impaired cell signaling and/or pro-inflammatory states. We postulate these moieties to be associated with poor outcome.
Methods: Eighty patients with decompensated cirrhosis were studied with 20 healthy controls. Proton nuclear magnetic resonance spectroscopy (NMRS) and reverse phase ultra-performance liquid chromatography tandem mass spectroscopy (UPLC-MS) and MS-MS fragmentation of plasma was performed. Orthogonal projection to latent structures discriminant analysis (OPLS-DA) was performed to detect biomarkers of 1-month mortality.
Results: Patients had a median (range) age of 55(23-75) years with 51 (64%) male and cirrhosis was alcohol-related in 40(50%). Median (range) MELD score was 14(6-40) and 1-month transplant free survival 76%. NMRS OPLS-DA accurately discriminated between patients and healthy controls (R2(X)=0.65, R2(Y)=0.84, Q2(Y)=0.76, cross-validated (leave-one out) sensitivity and specificity 100%, CV-ANOVA p<10-27). Metabolites increased in patients were lactate, tyrosine, and glucose with LDL, VLDL and phosphocholines being decreased in patients with cirrhosis. OPLS-DA for NMRS accurately discriminated between survivors and non-survivors (R2(X)=0.63, R2(Y)=0.64, Q2(Y)=0.41, AUROC 0.95 (95% CI 0.92-1.00), sensitivity 100%, specificity 79%, CV-ANOVA p<10-6) but did not correlate with hepatic encephalopathy (HE) grade. Metabolites increased in non-survivors included lactate, tyrosine and phenylalanine with lipid and phosphocholine resonances reduced in non-survivors. UPLC-MS-MS confirmed the following phosphocholines (PCs) and lysophosphatidylcholines (LPCs) contributed most to outcome prediction: LPC (16:0), LPC (18:2), LPC (20:0), LPC(22:6), PC (36:3), PC (36:2)(p<0.001 survivors v non survivors) with excellent predictive accuracy for electrospray injection (ESI)+ (AUROC 0.94 (0.87-0.98) and ESI- (AUROC 0.95(0.87-0.97; p<0.05 cf MELD score) mode profiles.
Conclusion: Plasma metabolic profiling with NMRS and UPLC-MS in patients with cirrhosis can accurately describe a metabolic phenotype of non-surviving patients but does not reflect HE grade; PCs and LPCs are highly discriminatory biomarkers of survival warranting validation in larger cohorts with targeted assays.

P25 VALIDATION OF A COMPUTERIZED PSYCHOMETRIC TEST FOR MINIMAL HEPATIC ENCEPHALOPATHY (MHE): THE SCAN TEST. PRELIMINARY RESULTS FROM A MULTICENTRE STUDY.

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Background: A computerized neuropsychological test, which is independent of familiarity with the alphabetical sequence and sensitive to MHE-related cognitive

alterations, would be useful for both clinical practice and international trials. The SCAN test seems to meet these requirements; however, it has not been validated internationally. A study has therefore been designed to evaluate its reproducibility across the world.
Material and methods: Five (Aarhus-Denmark, Barcelona-Spain, Naples/Rome/Padua-Italy) of the 10 centres that had agreed to participate provided preliminary data, for a total of 71 patients with cirrhosis (62% alcohol/alcohol plus virus-related), age (mean±SD) 60±19 years, MELD 11±4, overt HE ≤grade I according to the West Haven criteria. Patients underwent a semantic verbal fluency test ('animal naming test', i.e. the number of animals listed in 60 s) and the computerised SCAN test. Based on the SCAN test variables [reaction times (Rts), omission/commission errors], a summary SCAN index encompassing both time and accuracy was calculated [Rts weighted by omission errors; wRTs=RTs*(1+omission errors%/100)]. ANCOVA and multiple regression analysis adjusted for age and education levels were utilised for comparisons.
Results: The severity of cirrhosis was comparable across the five centres (MELD: $F_{4,66}=1.3$ $p=0.2$), even though a trend for more severe liver disease was observed in the Padua group, and significance was reached for the INR variable ($F_{4,66}=2.9$ $p<0.05$). The wRTs were found to be correlated with the INR ($r=0.27$ $p<0.02$), thus comparisons across centres were adjusted for the INR. No differences in wRTs were observed across centres $F(4,63)=0.9$ $p=0.45$. The wRTs were found to be correlated with the animal naming test.
Conclusion: These preliminary data suggest that the Scan test produces results which are: 1) comparable across five European centres; 2) related to liver function, and 3) related to a test of semantic verbal fluency, indicating that performance of the full, planned study is worthy.

P26 COGNITIVE IMPAIRMENT IN CIRRHOTIC PATIENTS: RELATIONSHIP BETWEEN CLINICAL VARIABLES AND MINIMAL HEPATIC ENCEPHALOPATHY

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Introduction: Minimal Hepatic Encephalopathy (MHE) is the more frequent complication of liver cirrhosis, being present in up to 60% of the patients depending on the method use for its detection.
Aim: Establishing what are the clinical variables associated to the presence of MHE.
Methods: 171 consecutive patients (Age= 63±12 yrs, Male=112, Meld=13,1±5.4) were considered eligible for the study in a 24-months-period in our Gastroenterological Unit. The West-Haven criteria, the clinical HE staging scale and a pool of standardized closed questions including the animal naming test were used to exclude overt HE. MHE was diagnosed when the Psychometric Hepatic Encephalopathy Score (PHES) was ≤ -4. Demographic data as well as a number of clinical and laboratory variables collected in each patient were analysed. A multiple regression analysis was used to identify the variables independently associated to the presence of MHE according to PHES.
Results: Eighty-six (50,2%) patients presented MHE according to PHES evaluation and no demographic differences were observed between MHE+ and MHE- patients. Comparing patients with and without MHE we found that they were statistically different in red blood cells count (3.7x106 vs 4x106, $p<0.01$), Child-Pugh Score (7.5 vs 6.8, $p=0.001$), history of overt HE (29/86 vs 11/85, $p<0.01$) , ascites (51/86 vs 29/85, $p<0.001$), mean arterial pressure (MAP), at ortho (87.4±10.6 vs 93.8±10.5, $p<0.001$) and clinostatic (87.9±10.5 vs 92.8±10.5, $p=0.003$) evaluations, albumin (3±0.6 vs 3.4±0.5, $p=0.001$) and sodium levels (135±5 vs 137±4, $p=0.007$), when the univariate analysis was performed. At a multivariate analysis, history of overt HE ($p=0,0449$) and

MAP at orthostatic evaluation (p=0,0055) were independently associated to the presence of MHE. Conclusions: Previous bouts of overt HE and a lower MAP are independently associated to MHE in cirrhotic patients.

P27 RENAL NKCC2 TRANSPORTER TRIGGERS ENHANCED URINARY AMMONIA EXCRETION DURING INDUCED HYPERAMMONEMIA: A NOVEL TARGET OF THERAPY FOR HEPATIC ENCEPHALOPATHY?

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Background and aims. The kidney plays a major role in the hyperammonemia seen after an upper gastrointestinal bleed in patients with cirrhosis. In the kidney, ammonia is synthesized by proximal tubular cells, after which 40-80% is reabsorbed in the medullary thick ascending limb (MTAL) of Henle's loop. Urinary renal ammonia excretion is mainly regulated by ammonia re-absorption via the co-transporter NKCC2 in the MTAL. NKCC2 accounts for more than half of the response of increased urinary ammonia excretion that occurs during chronic metabolic acidosis. This study aims at describing renal ammonia handling and expression of NKCC2 in a model of induced hyperammonemia.
Methods. Male rats were fed a hyperammonemic diet (mixture of amino acids mimicking hemoglobin) for 7 consecutive days (HD group). The control group was fed ad libitum (AL group). Arterial and renal vein blood was collected and para-amino hippuric acid was used to determine renal blood flow. The ureter was cannulated to collect urine samples. Expression of renal NKCC2 was measured using immunohistochemistry.
Results. The hyperammonemic diet successfully increased arterial ammonia levels in the HD group (HD-group: 85.4 (40.1-121.4) µmol/L and AL-group: 50.6 (20.0-114.4) µmol/L (p=0.04)). Total renal ammoniagenesis was significantly increased in the HD group [239.4.1(144.3-484.9) nmol/100bw/min vs 140.6(40.1-333.5) nmol/100bw/min]. Urinary ammonia excretion was 167.1 (25.8-411.9) nmol/100bw/min in the HD group and 58.5 (32.2-63.0) nmol/100bw/min in the AL group (p=0.03). The kidney increased the amount of ammonia excreted via the urine in the hyperammonemic HD-group (urinary ammonia excretion AL-group: 13.2 (3.7-49.9)% of total renal ammoniagenesis and HD-group 57.1 (23.8-66.5)%. Immunohistochemistry showed a marked overexpression of the renal NKCC2 in the HD group. The pH in the HD group was 7.35 (7.02-7.47) and 7.39 (7.33-7.45) in the AL group.
Conclusion. The kidneys are important in regulating ammonia homeostasis and respond by increased urinary ammonia excretion during hyperammonemia which is associated with increased expression of the NKCC2 co-transporter. NKCC2 may be a novel target for the treatment of hyperammonemia and subsequently hepatic encephalopathy.

P28 DEPRESSION, ANXIETY AND ALEXITHYMIA SYMPTOMS ARE MAJOR DETERMINANTS OF HEALTH RELATED QUALITY OF LIFE IN CIRRHOTIC PATIENTS

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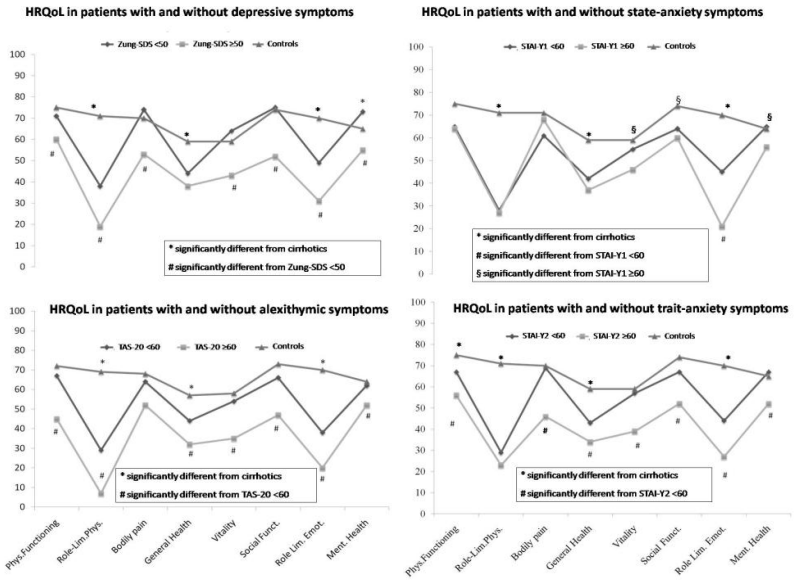
Background: Health related quality of life (HRQoL) is impaired in liver cirrhosis.
Aims: to establish the relevance of depression, anxiety, alexithymia symptoms and stage of liver failure on the patients' HRQoL.
Methods: Sixty cirrhotic patients (Age: 62±12; Sex (M): 42; Child-Pugh class (A/B/C): 29/23/8; MELD: 13±5) underwent an extensive neuropsychological assessment, including ZUNG-Self Depression Rating Scale (Zung-SDS), Spielberg State-Trait Anxiety Inventory Y1-Y2 (STAI Y1-Y2) and the Toronto Alexithymia Scale-20 items (TAS-20). Minimal hepatic encephalopathy (MHE) was detected by the psychometric hepatic encephalopathy score (PHES), HRQoL by Short-Form-36 (SF-36).
Results: symptoms of depression were detected in 34 patients (57%, 95%CI= 44-70%), state anxiety in 16 (27%, 95%CI=15-38%), trait anxiety in 17 (28%, 95%CI=17-40%) and alexithymia in 14 (31% 95%CI=16-46%). 22 cirrhotics had MHE (37%, 95%CI= 24-49%). All domains of SF-36 were impaired by each neuropsychological symptom. These symptoms were unrelated to the disease stage, hepatocellular carcinoma or MHE. A significant correlation was observed among each psychological test score and the summary components of SF-36. At multiple linear regression analysis including indices of liver failure (Child-Pugh and MELD scores), previous HE and the results of each psychological test as possible covariates, the mental component summary (MCS) of the SF-36 was significantly related to the results of TAS-20 (alexithymia) and ZUNG-SDS (depression) as well as to the Child-Pugh score; while STAI-Y2score (trait anxiety) was the only variable significantly and independently related to the physical component summary (PCS) of the SF-36 questionnaire. Neither depression nor anxiety and alexithymia symptoms were related to the patients' survival.
Conclusions: Depression, state and trait anxiety and alexithymia symptoms are frequent in cirrhotic patients and are among the major determinants of the altered HRQoL in these patients.

Figure. Impact of neuropsychological symptoms on HRQoL.



P29 SIMPLIFIED PSYCHOMETRIC HEPATIC ENCEPHALOPATHY SCORE PREDICTS THE OCCURRENCE OF OVERT HEPATIC ENCEPHALOPATHY IN CIRRHOTIC PATIENTS.

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Background: Hepatic Encephalopathy (HE) is a major complication of liver cirrhosis. As HE may be pharmacologically prevented, the identification of the patient at risk of developing overt HE is important.
Aim: to identify the predictors of overt HE in a large cohort of cirrhotic patients.
Patients/methods: over two years, 171 cirrhotics (age: 63±12; sex (M): 112; Child-Pugh-Class (A/B/C): 79/68/24; Meld: 13±6) without evidence of dementia (Mini Mental State Examination>26) and overt HE at enrolment (excluded by West Haven criteria and Chess score) were consecutively included in the study. Minimal HE was detected in 87 patients (50.8%) using the Symplified Psychometric Hepatic Encephalopathy Score (SPHES), including 3 of the 5 psychometric tests originally described (digit symbol, serial dotting, line tracing test). A history of previous bouts of overt HE was present in 40 patients (24%). During a mean follow-up of 10±7 months, 57 patients (33%) had at least one bout of overt HE.
Results: overt HE occurred in 47% of the patients with minimal HE and in 60% of those with previous HE. The presence of minimal HE increased the risk of overt HE of 3.88 times (C.I. 1.94-7.77), while previous HE increased the risk of overt HE of 4.98 times (C.I. 2.44-10.2). However, as 73% of the patients with previous HE also have minimal HE. This parameter together with age, Child-Pugh score, SPHES was included in a Cox multiple regression analysis. The only parameters independently related to the development of overt HE were the severity of liver failure (p=0.005) and minimal HE according to SPHES (p=0.002).
Conclusions: minimal HE and Child Pugh class are the main predictors of overt HE. About half of the patients with minimal HE are at risk of developing overt HE in the follow-up. Thus, in these patients a prophylactic treatment should be considered.

Cox multiple regression analysis: variables associated with the development of overt hepatic encephalopathy

	Risk-Ratio exp (β)	95% CI (β)	p Value
Age	1.008	0.98-1.03	ns
Child-Pugh score	1.26	1.07-1.49	0.005
MHE according to SPHES	2.62	1.43-4.8	0.002
Previous bouts of overt HE	1.56	0.88-2.78	ns

P30 MATHEMATICAL MODELLING OF AMMONIA METABOLISM IN CIRRHOSIS REVEALS THE IMPORTANCE OF ALTERATIONS IN SYSTEMIC AND SPLANCHNIC HEMODYNAMICS IN MODULATING ARTERIAL AMMONIA LEVELS

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Background & Aims. Hyperammonaemia occurs in patients with advanced cirrhosis, which is also associated with a major redistribution of blood flow (hyperdynamic circulation). Previous investigations have sought to explain hyperammonaemia on the basis of metabolic derangements. We hypothesized that haemodynamic disturbances associated with portal hypertension alone are sufficient to cause hyperammonaemia. To test this hypothesis, we developed a theoretical model, which predicts the resulting arterial ammonia levels when organ blood flow is modulated.
Methods. Assumptions on individual organ fluxes of ammonia (through the gut, liver, muscle, brain and kidney) were based on published arterio-venous differences and tracer kinetic data. In order to study the role of organ blood flow in isolation, we allowed hepatic detoxification function and ammonia production to remain normal. A wide range of conditions was investigated (increased portosystemic shunting, modulation of renal secretion). In addition, we used scenarios of organ blood flow corresponding to Child Pugh A, B and C.
Results. Without shunting, ammonia added to the renal vein determined arterial concentrations in a linear way. Hyperammonaemia developed when the portosystemic shunting fraction was more than 65%. The model predicted an elevation of bloodstream ammonia at each grade of cirrhosis (Child Pugh A, B and C) due to the redistribution of organ blood flow. Elevation of arterial ammonia was due to the combination of two factors: first the direct release of gastrointestinal production through the shunt, second the decreased hepatic detoxification due to the lower hepatic plasma flow.
Conclusions. Haemodynamic disturbances could cause the increase of bloodstream ammonia observed at each grade of cirrhosis. This can be partially attenuated by increased urinary ammonia excretion. In patients with cirrhosis, decreased hepatic functions and metabolic changes may further increase the arterial ammonia

P31 TYPE-B (ByPASS) MINIMAL HEPATIC ENCEPHALOPATHY IN PATIENTS WITH NON-CIRRHOTIC PORTAL HYPERTENSION: RELATIONSHIP WITH THE QUALITY OF LIFE.

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Introduction: Non-cirrhotic portal hypertension (NCPH) is an infrequent disease that causes portal systemic shunting. Type-B Hepatic Encephalopathy can originate from the presence of portal systemic shunts in absence of intrinsic liver disease.
Aims of the study: Investigating the presence of Type-B Minimal Hepatic Encephalopathy (MHE) and the relationship with Quality of Life (QoL) in a group of patients with NCPH.
Patients/Methods: 34 patients with NCPH and normal liver function (M=19, Age 48±14; Albumin 4±0.4 g/dl) and 27 Child-A cirrhotics (M=18, Age=69±7; MELD=9±2) without evidence of dementia (Mini Mental State Examination>26) were examined for MHE detection using the psychometric hepatic encephalopathy score (PHES), adjusted for age and education of healthy Italian population. In patients with NCPH the QoL was also

evaluated by SF-36 questionnaire corrected for age, education and occupation of healthy Italian population.
Results: MHE (diagnosed as a PHES ≤-4) was detected in 18% (95%CI 7-35%) of NCPH patients and in 30% (95%CI 14-50%) of Child-A cirrhotics (p=NS). In NCPH patients the presence of large varices, TIPS, previous bleeding and ascites were significantly more frequent than in cirrhotics. Patients with NCPH with or without MHE were similar in terms of age, education, liver function, presence of large varices or shunts. The results of PHES and SF-36 are reported in the Table. In MHE+ NCPH patients several domains of QoL were significantly impaired compared to MHE- and healthy controls.
Conclusions: Cognitive impairment can be detected in patients with NCPH and normal liver function probably because of the presence of large porto-systemic shunt suggesting the presence of Type-B MHE. Among the patients with NCPH a reduction of QoL can be observed only in those with MHE.

Table: PHES and SF-36 results in NCPH patients with and without MHE

	MHE + (n=6)	MHE – (n=25)	Control value (n 31)	P <
PHES	-6 ± 1.4	- 0.07 ± 2	-	0.001
Physical functioning	52.5 ± 24.4 *	76.4 ± 23.4	85.1 ± 12.4	0.001
Role limitation physical	45.8 ± 51 #	62 ± 39	78 ± 11	0.02
Bodily pain	52.7 ± 28.6 *	76.6 ± 25.8	80.9 ± 11.7	0.01
General health	37 ± 24.1	47.2 ± 17.6	65.6 ± 9.4°	0.001
Vitality	41.6 ± 14 *	57.6 ± 20	62.1 ± 7	0.007
Social functioning	54 ± 17 *	79 ± 24.6	77.2 ± 5.7	0.006
Role limitation emotional	50 ± 54.7	73.3 ± 36	75.6 ± 7.7	NS
Mental health	52.6 ± 16.3 *	69.7 ± 16.5	66.6 ± 6.3	0.01

*ANOVA or Student t test. *significantly different from MHE- and Control value at Newmans Keuls multiple comparison test; #significantly different from Control value at Newmans Keuls multiple comparison test; °significantly different from MHE- and MHE+ at Newmans Keuls multiple comparison test.*

P32 CEREBRAL MICROGLIA ACTIVATION IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION RELATES TO THE EXTENT OF CEREBRAL DYSFUNCTION – A [^{11}C]-PK11195 PET-STUDY

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Background: There is increasing evidence that an infection with the hepatitis C virus (HCV) may induce chronic fatigue and cognitive dysfunction. Data point to the fact that these neuropsychiatric symptoms occur in about half of the patients. Recent studies gave proof of virus replication within the brain and identified microglia and astrocytes as virus containing cells. We hypothesized that cerebral dysfunction in HCV-afflicted patients is associated with microglia activation.
Methods: Microglia activation was assessed by using [^{11}C]-PK11195 positron emission tomography (PET) combined with magnetic resonance tomography for anatomical co-registration of the binding capacity. For analysis of the [^{11}C]-PK11195 binding the reference tissue model was applied. [^{11}C]-PK11195-PET was performed in 22 patients with chronic HCV infection compared to 6 healthy controls. 12 patients were HCV-PCR positive (7 with neuropsychiatric symptoms and 5 without), 10 HCV-PCR negative (5

each with and without symptoms). Cognitive function was assessed by application of an extensive psychometric test battery. Presence of cognitive dysfunction was defined as an attention test sum score beyond the two standard deviation range of the healthy controls calculated from the results of the TAP (test battery for the assessment of attention from Zimmermann and Fimm). Presence of neuropsychiatric symptoms was defined as Fatigue Impact Scale (FIS) score above 45 points.
Results: Patients without attention deficits showed a significant accumulation of [^{11}C]-PK11195 in the amygdala, pons, pallidum and putamen ($p<0.05$) as well as in nucleus caudatus and thalamus ($p<0.01$). Patients with and without fatigue according to the FIS results did not differ significantly with regard to their PET results.
Conclusion: Patients without cognitive dysfunction showed significantly increased microglia activation with predominance in the basal ganglia while patients with cognitive dysfunction did not differ from controls. The result suggests that microglia activation has a neuroprotective function in HCV-infected patients.

P33 DIAGNOSTIC ACCURACY OF PSYCHOMETRIC TESTING FOR MINIMAL HEPATIC ENCEPHALOPATHY DETECTION IN CIRRHOTIC PATIENTS

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Introduction/aim: Minimal Hepatic Encephalopathy (MHE) is a neuropsychiatric syndrome peculiar of cirrhotics. MHE is detectable in 20-60% of cirrhotics depending on the tools utilized for the diagnosis, is associated with reduced quality of life, increased frequency of car accidents and traffic violations and is a risk factor for overt-HE. The Psychometric-Hepatic-Encephalopathy-Score-(PHES) is a recommended standard MHE diagnosis. A cut off of </=-4 discriminates the presence of MHE. The psychometric tests included in the PHES may be altered also in patients with cognitive impairment not due to liver cirrhosis.
Aims were to establish the sensitivity and specificity of PHES in diagnosing MHE and to compare the PHES accuracy to the simplified version SPHES which includes only 3 of the five tests originally used.
Patients/methods: 171 consecutive hospitalized cirrhotic patients without overt HE and signs of dementia (MMSE>26), 63±12-years, 112males, 123-viral aetiology, Child-Class-79/68/24(A/B/C), MELD score 13±5.5, 40 with previous episodes of overt-HE were studied with PHES and SPHES. 41-hospitalized subjects without liver disease and without dementia, (57±20years,19M) were studied as non-cirrhotic control group. Finally 44 healthy subjects, (31±12 years,16M) were used as healthy control group. ROC curve analysis was used to assess the diagnostic ability of PHES.
Results: PHES was abnormal in 86 out of 171 cirrhotic patients (50.3%), in 9 out of 41 non-cirrhotic controls (22%) and in none of the healthy controls (0%). The corresponding performance of SPHES was 51.4%, 19.5% and 0% respectively. Roc curve analysis was used to establish the sensitivity/ specificity of PHES. For this aim, non-cirrhotic and healthy-controls were pooled. AUC was 0.82. A cut-off PHES value of </=-4 has 50% sensitivity 89.4% specificity.
Conclusion: Both PHES and SPHES are simple tool to identify cirrhotics with cognitive impairment. A PHES cut off value of </=-4 has a sufficient specificity for detecting MHE.

P34 ALTERNATIVE TRANSCRIPTS OF GLUTAMINASE Gls2 GENE IN MAMMALIAN TISSUES

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Glutaminase (GA, E.C. 3.5.1.2) is expressed in most mammalian tissues and cancer cells, but the regulation of its expression is poorly understood. An essential step to accomplish this goal is the characterization of its species- and cell-specific isoenzyme pattern of expression. We demonstrate for the first time simultaneous expression of two transcript variants from the Gls2 gene in human, rat and mouse, using a combination of biochemical, molecular biological, and bioinformatics approaches. Short (LGA) and long (GAB) transcript forms were isolated in brain and liver tissue of human, rat and mouse. The short LGA transcript arises by a combination of two mechanisms of transcriptional modulation: alternative transcription initiation and alternative promoter. The LGA variant contains both the TSS and the alternative promoter in the first intron of the Gls2 gene. The human brain LGA transcript was cloned and fully sequenced. In vitro transcription and translation of human LGA yielded two polypeptides of the predicted size, but only the canonical full-length protein displayed catalytic activity. Interestingly, the relative abundance of GAB and LGA transcripts was species- and tissue-specific providing evidence of a differential regulation of Gls2 transcripts in mammals.
Supported by Grants: SAF 2010-17573 (Spanish Ministry of Science and Innovation), CVI-6656 (Regional Government of Andalusia) and RD06/0001/1012 (Spanish Health Institute Carlos III).

P35 CHANGE OF CEREBRAL GLUCOSE METABOLISM AND NEUROPSYCHIATRIC SYMPTOMS IN CHRONIC HEPATITIS C PATIENTS WITH ANTIVIRAL THERAPY

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Recently, antiviral therapy such as Interferon and Ribavirin combination is one of the most important treatment of chronic hepatitis C patients and widely used in the world. However, adverse effects of this therapy that Depression or neuropsychiatric symptoms make it difficult to be completed.
The aim of study is to evaluate neuropsychiatric symptoms with antiviral therapy and its correlation of effects on cerebral glucose metabolism (CMRglu) in chronic hepatitis C patients.
Seven patients with chronic hepatitis C undergoing antiviral therapy (Interferon and Ribavirin) were prospectively evaluated neuropsychiatric symptoms by neuropsychiatric test such as Digit symbol test(DST) , Block design test(BDT) ,and Self-rating Depression Scale(SDS) .
We assessed cerebral glucose metabolism (CMRglu) using [18F] deoxyglucose positron emission tomography (FDG-PET) before and the 8th weeks of treatment and after the therapy.
Compare to before and 8th weeks of treatment, SDS of all patients were worsened. CMRglu of six patients were 1-24% decreased in whole of the brain region. CMRglu of one patient was increased in the all of brain regions. There were no trend of result that DST and BDT before and 8th weeks of treatment. Compare to before and after the therapy, SDS of all three patients after the treatment were recovered within normal range. CMRglu of all of patients were 2-106% increased from 8th week of treatment in

whole of the brain. CMRglu of all of three patients were recovered and increased -8～73％from before the treatment.
These results suggest that antiviral therapy affects on cerebral glucose metabolism and Depression or neuropsychiatric symptoms in chronic hepatitis C patients. This depression or neuropsychiatric symptoms should be reversible. We believe that Cerebral glucose metabolism is affected by antiviral therapy and that might be reversible. It might be associated with depression or neuropsychiatric symptoms.

P36 TOLL LIKE RECEPTOR-4 ANTAGONIST PREVENTS ACETAMINOPHEN INDUCED ACUTE LIVER FAILURE IN MICE: A NOVEL THERAPEUTIC STRATEGY.

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Background and aim: Without transplantation, about 40% of patients with acute liver failure (ALF) die. Unregulated inflammation plays an important role in the pathogenesis. We hypothesised that Toll like receptor 4 (TLR 4) is critical in the progression of inflammation in ALF. The aims of the study were to determine whether 1) TLR4 antagonist in an acetaminophen (APAP) model of ALF in mice would prevent liver injury and end organ failure. 2) TLR4 KO mice are protected from the liver injury induced by APAP.
Method: Study 1: 3 groups of CD1 mice were studied (n=6 in each group) Naive, APAP, 500 mg/kg single dose IP after overnight fasting). APAP+TLR4 antagonist (STM28 (Osaka) 20ug IP, 1 hour before APAP and 6 hours later). Study 2: 3 groups of C57BL/6 mice were studied (n=6) in each group. Naive, APAP (500mg/kg single dose IP), APAP+TLR4 antagonist (IAXO, Innaxon) 3mg/kg, 1 hour before APAP and 6 hours later). Study 3: C57BL/6 TLR4 KO were administered APAP 500mg/Kg. Biochemistry and cytokines in plasma and tissue homogenate of liver, kidney and brain were measured. Brain water was measured using the dry-wet weight method.
Results: Both the TLR4 antagonist's (STM28 and IAXO compound) reduced the plasma liver enzymes, ammonia and creatinine to the control level. The increase in the plasma TNF-α induced by APAP (45±3.2) was attenuated following TLR4 antagonist (20±2.3) ($p<0.01$). This was associated with a reduction in brain water ($p<0.01$). Both the TLR4 antagonists significantly reduced liver necrosis. It showed an improvement in the survival as using the log rank test ($p<0.02$). TLR4 KO mice treated with APAP had significantly better survival than wild type controls ($p<0.002$).
Conclusion: These data provides evidence for an important role of TLR4 in APAP induced ALF and provide the rationale for a clinical trial of this strategy in ALF.

P37 PORTAL-SYSTEMIC EMBOLIZATION IS EFFECTIVE IN CHRONIC HEPATIC ENCEPHALOPATHY WITH CHILD-PUGH ≤7.

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Introduction: The embolization of large (>1 cm) portal-systemic shunts (EPSS) has been proposed in the treatment of chronic hepatic encephalopathy (HE) in patients with low risk of gastrointestinal bleeding. However, the experience is limited.
Aim: Assess the efficacy and safety of EPSS.
Methods: A retrospective study (1998-2011) was performed in our hospital. A favorable response was defined as: increasing autonomy + marked decrease in the number of episodes of HE + more than 6 months follow-up.

Patients: 15 patients with hepatic cirrhosis were submitted to EPSS because of chronic HE (recurrent or persistent).
Results: No severe early complications were described after the EPSS. Nine patients had a favorable response and 6 a poor outcome. Among patients with favorable response 4 relapsed (1.6 episodes of HE per patient) which caused in total 6 days of hospitalization during 42±40 months of follow-up. In the group of poor outcome, all the patients relapsed (average of 8 episodes per patient) and 3 deaths were registered before completing 6 months of follow-up. This group needed in total 183 days of hospitalization during follow-up (11±10 months). Only one patient had a gastrointestinal bleeding 4 years after EPSS. The Child-Pugh was a good predictor of favorable response with a cut-off ≤7 points (ROC curve: sensitivity 88.9 %, specificity 83.3%). Patients with Child-Pugh ≤7 (n= 9) had a favorable outcome, except for one patient (83 years). Patients with Child-Pugh >7 had poor outcome after EPSS, except for one alcoholic patient with Child-Pugh=8.
Conclusions: Embolization of large portal-systemic shunts is a safety process in patients with chronic HE and low risk of variceal bleeding, and offers clear benefits in patients with a Child-Pugh score ≤7.

P38 INCREASE OF ARGININE UPTAKE IN ASTROCYTES PREINCUBATED WITH AMMONIA OR GLUTAMINE IS ABOLISHED BY Y+LAT2 SILENCING.

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Increased astrocytic uptake of arginine (Arg) contributes to increased NO synthesis and oxidative/nitrosative stress in ammonia-exposed CNS tissues. A previous analysis indicated that, by kinetic criteria, the increased uptake is to be solely related to the increased expression and function of the heteromeric y+LAT2 transporter, which mediates Arg/glutamine (Gln) exchange (Zielińska et al., 2012). To directly confirm this supposition we compared the effects of ammonia and Gln (48h, either compound 5 mM) on Arg uptake by 4 different Arg-transporting systems (B0+, b0+, y+L, y+), in astrocytes pretreated or not, with siRNA complementary to y+LAT2 mRNA. Treatment with siRNA confirmed the dominating role y+LAT2 in Arg uptake in astrocytes and abolished stimulation of the uptake by ammonia. Preincubation with Gln increased the Vmax of Arg uptake in astrocytes in y+LAT2-non-silenced astrocytes, and this stimulatory effect disappeared following the silencing. Similarity of the effects of ammonia and Gln indicates that y+LAT2-mediated increase of Arg uptake in exchange for Gln may be one of the mechanisms by which Gln accumulating intracellularly contributes to ammonia-induced nitrosative stress.
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P39 MORPHOFUNCTIONAL CHANGES IN AN EXPERIMENTAL MODEL OF MINIMAL HEPATIC ENCEPHALOPATHY

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Prehepatic portal hypertensive rats due to partial portal vein stricture displays morphofunctional changes and induce a minimal hepatic encephalopathy (MHE). The aim of this study was to evaluate in a rat MHE model morphofunctional features of different neural cells focusing in brain cortex and hippocampal CA1.
Methods: Two groups of rats were used: MHE and Sham operated. Astrocytes were studied by immunolabelling with the glial fibrillary acidic protein (GFAP) and S100β protein. Neurons were assessed with neuronal nuclear marker (NeuN), microtubule associated protein-2 (MAP-2) and neuronal filaments 200 kDa (Nf-200). Capillaries were immunolabelled with Nestin. The hypoxia inducible factor -1α (HIF-1α), a transcription factor involved in oxygen homeostasis, and two of it downstream proteins, the P-glycoprotein (P-gp) and the erythropoietin receptor (Epo-R), were evaluated in both brain areas.
Results: GFAP showed an increased area and number of hippocampal astrocytes but not in the cortex of MHE animals, meanwhile S100 β was increased in both brain areas. Neuronal number did not differ in the studied areas, but MAP-2 and Nf-200 were significantly decreased in both areas. The neuronal cytoskeleton showed qualitative and structural changes in the cerebral cortex of the MHE group. Increased capillaries area in MHE animals, Nestin-immunoreactivity, in the hippocampal CA1 area was also observed. We also found high expression of HIF-1 α in cortex neurons of the MHE animals. P-gp and Epo-R expressions are in agree with the HIF-1 α result. The hypoxic tissue state was not accompanied with hypoxemia.
Conclusion: Neuronal, astroglial and capillaries morphofunctional changes in a model of MHE are here presented. It could be proposed that a hypoxic tissue state is developing by occupying the binding domain of HIF-1 α, through ammonia, manganese or both, thereby avoiding its degradation and inducing its stabilization leading to the overexpression of P-gp and the Epo-R.

P40 MINIMAL HEPATIC ENCEPHALOPATHY AND DISEASE SPECIFIC QUALITY OF LIFE IN OUTPATIENTS WITH CIRRHOSIS: PRELIMINARY RESULTS FROM A PROSPECTIVE COHORT

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Background: The extent to which minimal hepatic encephalopathy (MHE) affects health-related quality of life (HRQoL) is debated.
Methods: A prospective cohort study including outpatients with cirrhosis from two Danish liver clinics. Patients with clinically overt HE (Westhaven Criteria Grade 1 to 4) were excluded. Patients were evaluated for MHE using the continuous reaction time test. HRQoL was assessed using the Chronic Liver Disease Questionnaire (CLDQ). The CLDQ score was classed as low (1.00 to 3.99 points) or high (4.00 to 6.00 points) with higher scores indicating a better quality of life. Logistic regression analysis using binary

outcomes with results reported as odds ratio (OR) was used to assess the potential correlation between MHE and low CLDQ-scores.
Results: In total, 76 patients were included and followed for a mean duration of 22 months. Most patients had cirrhosis due to alcoholic liver disease (89%). More than half of the included patients (52%) showed signs of MHE. The mean CLDQ score was 4.40 ± 0.69 (range 2.7-5.9). The proportion of patients with a low CDLQ score was 24%. There was a clear correlation between a low CLDQ score and the Child-Pugh Score at baseline, (OR = 1.62; 95% CI = 1.07-2.45), but not with MHE (OR = 2.04; 95% CI = 0.53-7.77). Conclusion: In the present study, a low HRQoL was positively correlated to the Child-Pugh score, but not with MHE. In the present population, the overall severity of liver disease, but not MHE, was an indicator of poor HRQoL.

P41 EFFECTS OF ORNITHINE PHENYLACETATE IN PATIENTS WITH CIRRHOSIS AND UPPER GASTROINTESTINAL BLEEDING.

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Introduction: Ornithine phenylacetate (OP) is a new drug that is being evaluated for hyperammonemia and related hepatic encephalopathy (HE) in cirrhosis. OP has been shown to be safe in healthy subjects and stable patients with cirrhosis.
Aim: An open-label, dose-escalating, single cohort study was performed to confirm the safety and tolerability of OP in decompensated cirrhotic patients with upper gastrointestinal bleeding (UGB).
Patients: 6 patients (5 males, 1 female) without signs of HE and normal renal function were included within 24 hours of UGB.
Methods: OP was administered as continuous infusion up to 10 g/24 h (0.42g/h) for 5 days. The infusion was started at 33% of target dose and increased at 12 h intervals to 100% of target infusion rate at 24 h.
Results: No severe adverse events (AE) were observed. Mild AE (< 1 day) were reported in 3 patients: nausea (n=4), headache (n=2) and abdominal pain (n=1). One unrelated to OP, 4 unlikely related and 2 possibly related. No patients developed complications related to cirrhosis (no episodes of HE were reported), patients received full dose. Plasma ammonia (baseline: 68±22, 24 h:49±15; 48h:48±14, 72h:40±8, 96h:30±11; 120 h:33±14; microM) showed a progressive drop (ANOVA repeated measures $p<0.01$ between baseline and 72h, 96h and 120h). Phenylacetylglutamine (PAGN) in urine showed a progressive rise during the study, achieving an accumulated excretion at the end of day 5 of 51±45 mmol. The concentration of ornithine increased progressively (+220% at day 3) while glutamine showed a significant decrease (-56% at day 3).
Conclusions: OP is a safe and well tolerated drug in patients with cirrhosis and UGB. OP administration is associated with a marked drop of plasma ammonia and the appearance of PAGN in urine. OP may be useful in cirrhosis to treat or prevent hyperammonemia and related HE secondary to UGB.

P42 NEUTROPHIL INTRACELLULAR TOLL-LIKE RECEPTOR (TLR9) EXPRESSION SERVES AS A BIOMARKER THAT DETERMINES PRESENCE AND SEVERITY OF ENCEPHALOPATHY IN ACUTE LIVER FAILURE AND CIRRHOSIS

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Background and aim: Patients with acute liver failure (ALF) and cirrhosis are prone to sepsis and inflammation which may hasten the development of hepatic encephalopathy (HE) and cerebral oedema. The relationship between neutrophil dysfunction, an important biomarker of poor outcome in liver failure, and the development of HE is unclear. Provisional data from our group suggests that TLR9 could be an important prognostic biomarker that differentiates ALF patients that go onto develop HE. The aim of the study was to therefore characterize the relationship between neutrophil TLR9 and HE.

Methods: In healthy controls (n=12), patients with ALF (n=12) and cirrhosis (n=50) we investigated neutrophil TLR9 expression using fluorochrome-conjugated monoclonal antibodies [CD16(PE)/CD11b(APC-Cy7)/TLR9(APC)] by flow cytometry. We determined respsonses to endotoxin and bacterial challenge at baseline, and following 2-hour stimulation with lipopolysaccharide(2ug/ml) and ammonia(400uM).Plasma and intracellular cytokine production were measured.

Results: Baseline neutrophil TLR9 expression was significantly higher in patients with HE (ALF: overall significance $p<0.05$; grade-3/4 versus controls: $p<0.02$, versus grade-0/1/2: $p<0.03$) (Cirrhotics: overall significance $p<0.05$; grade-3/4 versus controls: $p<0.03$, versus grade-0/1/2: $p<0.05$). Moreover, baseline TLR9 expression was associated with HE severity and higher IL6 and IL8 levels. CD16 expression was downregulated by a median of 45% (range 25-85%) in ALF patients with grade-3/4 HE compared to controls and in cirrhotics by 88% (range 5-90%) (grade-3/4 versus controls: $p<0.05$). Exposure to lipopolysaccharide and ammonia upregulated TLR9 and downregulated CD16 expression.

Conclusion: Neutrophil TLR9 expression in patients with liver disease serves as useful biomarker that differentiates those who develop high-grade HE from those who do not. High baseline TLR9 expression and low-CD16 expression promote a pro-inflammatory cytokine milieu that may help to explain the propensity to develop infection and why inflammation hastens the development of HE. TLR9 antagonists may be of therapeutic value in restoring neutrophil activity.

P43 FEATURE OF MINIMAL HEPATIC ENCEPHALOPATHY

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Aims: Neuropsychological Test (NP-Test), composed of 8 function tests, is a convenient method for diagnosing Minimal hepatic encephalopathy which does not require a specific location or a professional examiner. Our aim was to investigate the relations among Minimal hepatic encephalopathy and hepatic functional reserve, nutritional status, and Quolity of life (QOL).

Methods: A total of 44 liver disease patients was evaluated by NP-Test. The NP-test score of 0 was considered to be normal and 8 to be the worst. They were also requested to fill up the questionnaires for sleeping and QOL (composed with Pittsburgh Sleep Quality index（PSQI), Epworth Sleepness Scale (ESS), cirrhosis symptomatic score (CSS), and the SF36, which is a multi-purpose, short-form health survey. Hepatic functional reserve was evaluated in their blood. Respiratory quotient (RQ) and resting energy expenditure (REE) were measured using indirect calorimetory. Statistical analyses were performed using Mann-Whitney U test.
Results: Among all the patients, Only 6 cases (13.7%) scored normal on NP-Test. The median value of NP-Test in the patients was 2 (ranged 0 to 8). Next we divided 44 patients into two groups, a group which scored 2 and under (group 1) another group with over 3 (group2). General health perceptions and mental health tested in SF36 and Prothrombin time (PT) showed significantly low score in the group2 ($p=0.02$, $p=0.01$, $p=0.01$, respectively). Also Child-Pugh score was significantly high in the group 2 ($p=0.02$). Furthermore, values of NP-test significantly correlated with serum cholinesterase ($r= -0.363$, $p=0.03$), total protein ($r= -0.497$, $p=0.002$), PT($r= -0.350$, $p=0.02$) and Child-Pugh score($r= 0.347$, $p=0.04$). Meanwhile, the score of PSQI, ESS, CSS, and SF36 did not significantly associate with the NP-test score. Neither RQ nor % REE was related to the scores of NP-Test.
Conclusions: Hepatic functional reserve related closely to the severity of Minimal hepatic encephalopathy.

P44 BLOOD AND BRAIN ADMA CONTENT AND THE EXPRESSION/ACTIVITY OF ADMA METABOLIZING ENZYMES IN BRAIN METABOLISM ARE ALTERED IN THIOACETAMIDE INDUCED-LIVER FAILURE BUT NOT IN SIMPLE HYPERAMMONEMIA.

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Neurotoxic effects of ammonia are a key causative factor in HE. However, earlier studies disclosed substantial differences between the responses of blood and brain parameters to HE associated with liver failure induced with thioacetamide (TAA) and simple hyperammonemia (HA) (Hilgier et al., 1996, and references therein). Recent evidence suggests the increase of dimethylarginine (ADMA) in brain to be one of the causes of impaired NO synthesis in HE (Balasubramaniyan et al., 2011). Here we show that exposure of rats to TAA specifically plasma and brain ADMA levels, decreased the activity and expression of the ADMA-degrading enzyme, dimethylargininedimethylaminohydrolase (DDAH1), and was associated with the tendency towards increase of the expression of the ADMA-synthesizing enzyme, protein arginine methyltransferase (PRMT1). None of the above parameters was altered in simple hyperammonemia based on i.p. administration of ammonium acetate. The results underscore the role of factors other than ammonia in the aspects of the pathogenesis of HE related to impaired NO synthesis.
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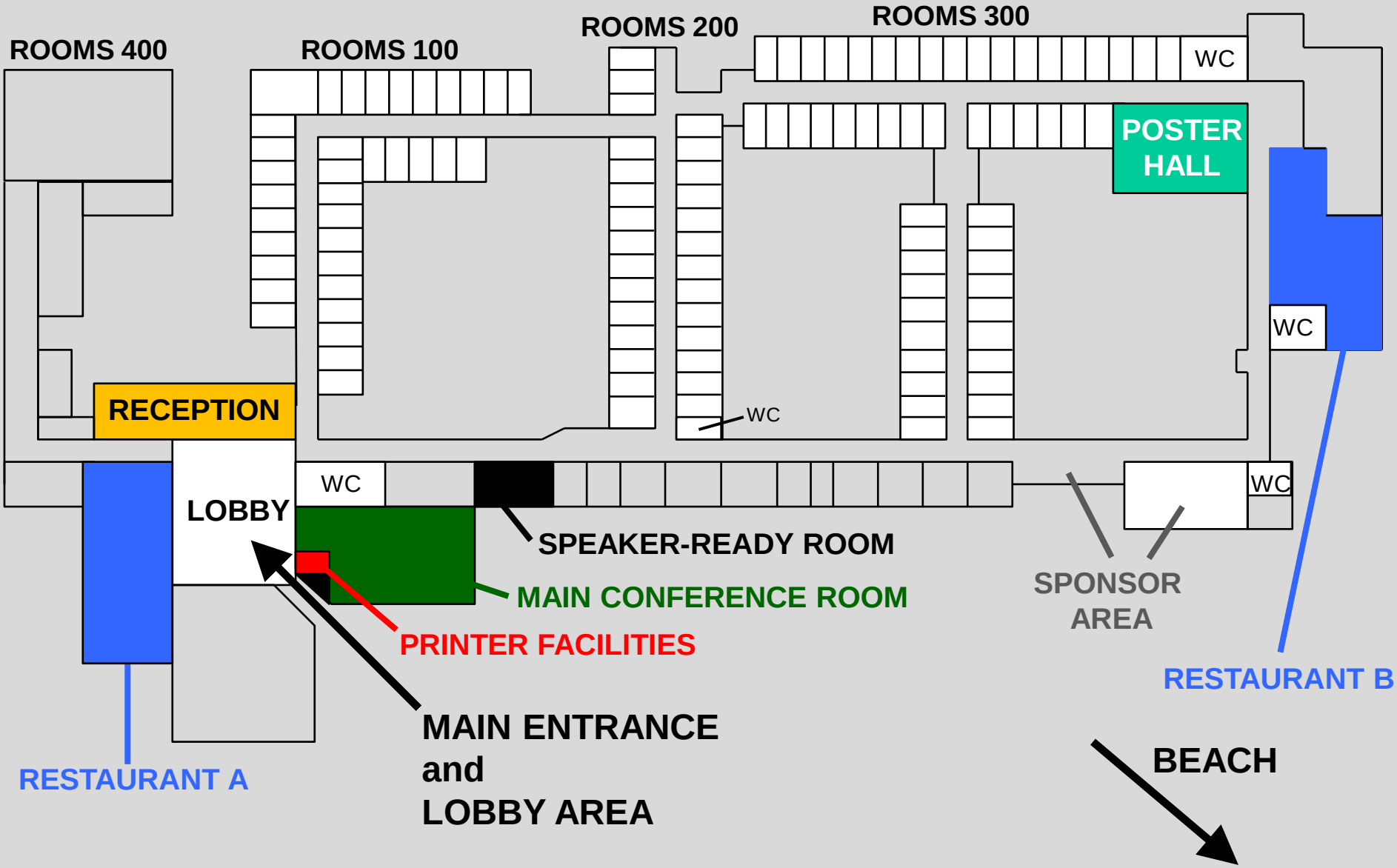
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SPONSOR AREA
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RESTAURANT A
MAIN ENTRANCE and LOBBY AREA
BEACH