It is with great pleasure I welcome you to the 16th ISHEN meeting in London. The understanding of the pathophysiological basis of hepatic encephalopathy has gained enormously from the very close interaction between basic scientists, translational biologists and clinicians. ISHEN, which was born over 40 years ago, has been at the forefront of creating an environment for these interactions. From a complication of cirrhosis which was thought to be mediated by alterations in nitrogen metabolism, the understanding the condition has progressed to involve multiple pathways including inflammation, alterations in brain neurochemistry and the involvement of many cell types in its pathogenesis. This understanding is rapidly evolving into new diagnostic and treatment paradigms. The 16th ISHEN meeting in London has been developed to explore these novel ideas in the understanding of hepatic encephalopathy keeping in mind how these advances may be relevant to the patient at present, in the near future and in the long-term. The first day is about what we know, days 2 and 3 is about new and exciting developments in the pathogenesis of hepatic encephalopathy and day 4 will focus more on the clinical aspects.

As with all successful scientific organisations, we have developed the programme keeping in mind the importance of discussion and engagement of young scientists and clinicians. In answer to our call, we are proud to announce that a record number of abstracts (nearly 90) will be presented at this meeting and we are offering about 51 full bursaries. As an organization, ISHEN is committed to its young members. For the first time, we have introduced workshops, which would allow closer and more focused discussions.

The 16th ISHEN meeting will celebrate the lives of two great scientists and clinicians, Professors Andy Blei and Professor Juan Cordoba who passed away prematurely in the past couple of years. Their contributions to ISHEN are enormous and their human values exemplary. The committee has decided to hold the 1st Andy Blei lecture, which will run in perpetuity. Professor Roger Butterworth, a long-standing friend of Andy’s, has kindly agreed to deliver the lecture. A memorial will be held in the memory of Professor Cordoba. The young investigator prize will carry his name now and in the future.

I invite you to the meeting to participate, contribute, debate, share your knowledge, interact with the best scientists in the field and most of all have a fantastic time.

Rajiv Jalan
President, ISHEN
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<tr>
<td>12:30 – 13:20</td>
<td>Lunch</td>
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<td>Steam Bake and Grill Restaurant</td>
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<tr>
<td>15:30 – 16:00</td>
<td>Refreshment Break</td>
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<tr>
<td>16:00 – 17:30</td>
<td>What we Know: Clinical Science (part1)</td>
<td>Kevin Muller, Hendrik Vilstrup</td>
<td>16:00 - Marsha Morgan, 16:20 - Tarek Hassanein, 16:40 - Gerald Kircheis, YI Talks: 17:00 - Rune Gangsøy Kristiansen, 17:15 - Carmina Montoliu</td>
<td>Sharpe Suite</td>
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<tr>
<td>17:30 – 17:45</td>
<td>Refreshment Break</td>
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**THURSDAY 11th SEPTEMBER 2014**

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<tr>
<td>08:30 – 10:00</td>
<td>Workshop 1: Basic Science:</td>
<td>Freimut Schliess, Marc Oria</td>
<td>YI Presenters</td>
<td>Sharpe Suite A</td>
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<td>08:30 – 10:00</td>
<td>The Cellular Basis of HE</td>
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<td>10:00 – 10:30</td>
<td>Workshop 2: Clinical Science: Targeting</td>
<td>Debbie Shawcross, Jasmohan Bajaj</td>
<td>YI Presenters</td>
<td>Sharpe Suite B</td>
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<td>the gut/liver/brain Axis</td>
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<td>12:30 – 13:30</td>
<td>Lunch Break</td>
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<td>Steam Bake and Grill Restaurant</td>
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<td>15:30 – 16:00</td>
<td>Refreshment Break</td>
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<tr>
<td>16:00 – 17:30</td>
<td>Ammonia: More than just a Neurotoxin</td>
<td>Peter Ott, Chris Rose</td>
<td>16:00 - Srinivasan Dasarathy, 16:20 - Rajeshwar Mookerjee, 16:40 - Alexander Thrane, YI Talks: 17:00 - Balasubramaniyan Vairappan, 17:15 - Veronika Rackayova</td>
<td>Sharpe Suite</td>
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<tr>
<td>17:30 – 17:45</td>
<td>Refreshment Break</td>
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<tr>
<td>17:45 – 18:30</td>
<td>Andrés Blei Lecture</td>
<td>Rajiv Jalan</td>
<td>Roger Butterworth</td>
<td>Sharpe Suite</td>
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<tr>
<td>19:00 – 19:45</td>
<td>Football Match – Europe vs Rest of the World</td>
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<td>Steam Bake and Grill Restaurant</td>
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**FRIDAY 12th SEPTEMBER 2014**

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<tr>
<td>08:00 - 10:00</td>
<td>Workshop 3: Basic Science: Brain Swelling as an Endpoint</td>
<td>Chris Rose Arthuir Cooper</td>
<td>YI Presenters</td>
<td>Sharpe Suite A</td>
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<tr>
<td>08:00 - 10:00</td>
<td>Workshop 4: Clinical Science: Quantifying &amp; Qualifying MHE</td>
<td>Karin Weissenborn Marsha Morgan Gerald Kircheis Sara Montagnase Piero Amodio</td>
<td>YI Presenters</td>
<td>Sharpe Suite B</td>
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<tr>
<td>10:00 - 10:30</td>
<td>Refreshment Break</td>
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<tr>
<td>10:30 - 12:30</td>
<td>The Microbiome in Liver Disease</td>
<td>Nathan Davies Alastair Forbes</td>
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<td>Sharpe Suite</td>
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<td>12:30 - 13:30</td>
<td>Lunch Break</td>
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<td>15:30 - 16:00</td>
<td>Refreshment Break</td>
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<td>16:00 - 17:50</td>
<td>Signalling and the Blood Brain Barrier</td>
<td>Jan Albrecht Scott Nyberg</td>
<td>16:00 - Peter Searson 16:25 - AR Jayakumar 16:50 - Vicente Felipe YI Talks: 17:20 - Matthew McMilin 17:35 - Laia Chavarria</td>
<td>Sharpe Suite</td>
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<td>17:50 - 18:00</td>
<td>Refreshment Break</td>
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<tr>
<td>18:00 - 18:30</td>
<td>In Memory of Prof. Juan Cordoba</td>
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<td>19:30 - 00:30</td>
<td>Medieval Gala Dinner and Prizes</td>
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<td>Northcote House</td>
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**SATURDAY 13th SEPTEMBER 2014**

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<tr>
<td>08:00 - 10:15</td>
<td>Where are we going? i. Translational Research</td>
<td>Marsha Morgan Piero Amodio</td>
<td>08:00 - Kevin Mullen 08:20 - Hendrik Vilstrup 08:40 - James Orr 09:00 - Sara Montagnase 09:20 - Alistair Lee</td>
<td>Sharpe Suite</td>
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<tr>
<td>10:15 - 10:45</td>
<td>Refreshment Break</td>
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<tr>
<td>12:45 - 13:30</td>
<td>General Membership Meeting &amp; Final Remarks from Current and Future ISHEN Presidents</td>
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<td>Sharpe Suite</td>
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<tr>
<td>13:30</td>
<td>Lunch &amp; Departures</td>
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<td>Steam Bake and Grill Restaurant</td>
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**ENDORSEMENTS**

ISHEN 2014 has received endorsements from the following affiliated organisations.
Abbott’s Established Pharmaceuticals Division (EPD), headquartered in Basel, Switzerland, is focused on helping make the world a healthier place by bringing the benefits of trusted pharmaceutical brands to much broader patient populations - in the developed world and, particularly, in emerging markets. EPD brings value to its patients and customers through deep localization and a growing portfolio of high-quality established pharmaceutical products.

Abbott is a global healthcare company devoted to improving life through the development of products and technologies that span the breadth of healthcare. With a portfolio of leading, science-based offerings in diagnostics, medical devices, nutritional and branded generic pharmaceuticals, Abbott serves people in more than 150 countries and employs approximately 70,000 people.

For more information www.abbott.co.uk

Merz Pharma is an innovative and international healthcare company with its own research and development department. The company is bundling the activities of its subsidiaries Merz Pharmaceuticals, Merz Consumer Care, and Merz Dental, with products ranging from prescription drugs and OTC products for health and wellness, materials for dentists and dental technicians to hygienic products for medical disinfection and cleaning.

For more information - www.merz.com

Founded in 1996, Norgine is an independent pan-European specialty pharmaceutical company. Throughout our long history, we have sought to develop and market high quality and innovative products that patients, healthcare professionals and budget holders want and value. We are focused on maintaining our position as a leading pan-European specialty pharmaceutical company. Through creative and flexible partnering, we will continue to drive the growth of our product portfolio and foster our pipeline. In addition to our leading products, XIFAXAN® 550, MOVICOL®, MOVIPREP®, Norgine has a strong portfolio of development projects, principally in the areas of gastroenterology, hepatology and supportive care.

For more information - www.norgine.com

Ocera Therapeutics, Inc. is a clinical stage biopharmaceutical company focused on the development and commercialization of OCR-002 (ornithine phenylacetate). OCR-002 is an ammonia scavenger which has been granted Orphan Disease and Fast Track status from the FDA to treat hyperammonemia and associated hepatic encephalopathy in patients with liver cirrhosis, acute liver failure and acute liver injury.

For information, please see www.ocerainc.com

Salix Pharmaceuticals is a specialty pharmaceutical company that offers innovative gastroenterology treatments.

We are committed to licensing, developing, and marketing products to treat gastroenterology disorders. It is our mission to give healthcare professionals and patients the most effective solutions in gastroenterology.

For more information - www.salix.com

Science has been at the heart of Yakult since its probiotic drink was developed in 1935. The company sponsors independent researchers in hospitals, universities and institutes throughout the world. Over 100 human studies on Lactobacillus casei Shirotia have now been published in peer-reviewed papers.

For more information at www.yakult.co.uk/hcp or contact science@yakult.co.uk for free educational resources.
Agustín Albillos
Agustín Albillos is Head of the Department of Gastroenterology and Hepatology at Hospital Universitario Ramón y Cajal, and Professor of Medicine at the University of Alcalá, Madrid, Spain. He has been working in the field of cirrhosis and its complications, since his postdoctoral fellowship at Prof. Bosch and Prof. Groszmann’s laboratories. He is President Elect of the Spanish Association for the Study of the Liver, and member of EASL and AASLD.

Jan Albrecht
Education: 1986 – M Sc. (Biology) University of Warsaw, 1970 – Ph. D. (Biochemistry) University of Leiden. Position: Professor and Head, Department of Neurotoxicology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland. Memberships: European Academy, Polish Academy of Sciences, Editorial Boards; among others: Journal of Neurochemistry, Neurochemical Research (Associate Editor).

Piero Amodio
Piero Amodio was born on June the 11th 1953 in Udine, Italy. He lives in Padova. E.mail address: piero.amodio@unipd.it
Education:
1978 specialization qualification (110/110 cum laude) in Medicine at the University of Padova with the thesis “Study on the renal proximal tubular function in cirrhosis by means of urinary enzymes measuring”.
1982 Specialised in Respiratory Medicine (70/70 cum laude) with the thesis “Lung metabolism of PGE2 and PGF2α in liver cirrhosis”. 1987 Specialised in Internal Medicine in (70/70 cum laude) with the thesis “Carnitine metabolism in liver cirrhosis.
1993: 6th European Educational Programme in Epidemiology, Florence.
1997: Grant CNR/FNRS) for research at the “Unité d’Exploration Electrophysiologiques du Systeme Nerveux” of the Catholic University of Bruxelles.
Professional Activity:
From 1978 to 1988 Medical Doctor in the Dpt of Clinical Medicine of the University of Padova.
From 1988 Researcher in the Dpt of Clinical Medicine of the University of Padova.
Responsible of the Service for Cognitive Dysfunctions in Internal Medicine Disorders, Clinica Medica 5, Padova
From 2007 Ag. Professor of Internal Medicine
2010 Director of the Interdepartmental Research Centre for Neuropsychiatric Disorders in Internal Medicine (CIRMANMEC), University of Padua
Member of:
- the Italian Society of Internal Medicine, Secretary of its Veneto Section
- the Italian Society of Clinical Neurophysiology,
- the Italian Association of the Study of the Liver.
Head of the National Commission for the Study of Hepatic Encephalopathy of the Italian Association for the Study of the Liver.
- The European Association for the Study of the Liver
- The Governing Board of the Italian College of Internal Medicine
- The International Society for Hepatic Encephalopathy and Nitrogen Metabolism

Dr. Javier Ampuero
Dr. Javier Ampuero earned his Medical degree in Córdoba (Spain) in 2009. Later, he did his residence in Gastroenterology (2010-2014). During 2013, he was in the Hospital of the University of Pennsylvania (Philadelphia, United States) for three months. Currently, he works at Unit for the Clinical Management of Digestive Diseases in Valme University Hospital (Sevilla, Spain). His primary project has focused on Hepatic Encephalopathy under the mentorship of Dr. Manuel Romero-Gómez. In particular, they are focusing on the role of metformin on spectrum of Hepatic Encephalopathy, as they demonstrated the protective role of this drug. They are carrying out a randomized clinical trial to demonstrate the impact of metformin on cirrhotic patients. In fact, this topic is going to be the focus of his doctoral thesis (in September 2014). On the other hand, they are researching about the role of genetic factors on the methods to detect Minimal Hepatic Encephalopathy. In addition, he has completed a Master of Statistics in Science Research Methodology, what has given him the opportunity to be able to design different studies and perform a variety of statistical analysis. These other researches include topics about Hepatitis C, Hepatocellular Carcinoma, Non-Alcoholic Fatty Liver Disease, and Inflammatory Bowel Disease.

Djillali Anne
Djillali Anne was appointed Professor of Medicine: Critical Care Medicine at the University of Paris in 1996 having completed his PhD in Pharmacology in 1995 and MD in 1991. Professor Anne also has Specialist Qualifications in Intensive Care Medicine, Cardiologist, Internal Medicine, Statistics and Pharmacology. Professor Anne is the Director of the 36-bed intensive care unit at Raymond Poincaré, Assistance Publique Hôpitaux de Paris (APHP) which is the premiere tertiary care hospital in France. He is Chief Counsellor of the Minister of Health for medical education and research, Dean of the School of Medicine at University of Versailles Saint Quentin and was President of the French Society of Intensive Care (SRLF) from 2011 to 2013. Professor Annane’s main research interests are the pathophysiology and management of septic shock and he has been involved as the coordinator / principal investigator of several large multi-national randomized controlled trials. He has published more than 300 peer-reviewed articles.

Prof Michael Arthur
Professor Michael Arthur is President and Provost of University College London. Prior to this he was Vice-Chancellor of the University of Leeds, and formerly Professor of Medicine (1992), Head of the School of Medicine (1998-2001) and Dean of the Faculty of Medicine, Health and Life Sciences in Southampton (2003-04). He is a hepatologist with research interests in liver cell biology developed initially at the University of California, San Francisco (1986-1988) and more recently as a Fulbright Distinguished Scholar at Mount Sinai School of Medicine in New York (2002). Professor Arthur became a Fellow of the Academy of Medical Sciences in 1998. Professor Arthur has a significant national and international profile. He was Chair of the Advisory Group for National Specialised Services (NHS) (2010-2013) and is a member of the Council of Worldwide Universities Network and the Russell Group of Universities. Professor Arthur took up his current position on 1 September 2013.

Jasmohan S. Bajaj
MBBS, MD, MS, FACC
Jasmohan S. Bajaj, MBBS, MD, MS, is Associate Professor of Medicine, Division of Gastroenterology, Hepatology, and Nutrition at Virginia Commonwealth University and McGuire VA Medical Center in Richmond, VA. He is a Fellow of the American Gastroenterological Association and American College of Gastroenterology. Dr. Bajaj earned his MBBS from Delhi University at Maulana Azad Medical College. He completed an internship in internal medicine at Delhi University at Maulana Azad Medical College and internal medicine residency at the State University of New York Health Science Center in Brooklyn. He furthered his medical training with a fellowship in gastroenterology and hepatology at the Medical College of Wisconsin Affiliated Hospitals, then went on to earn an MS in epidemiology at the Medical College of Wisconsin in Milwaukee. Active in research, Dr. Bajaj has served as a principal investigator or co-investigator for numerous clinical trials in areas such as hepatic encephalopathy, chronic liver disease and microbiome. Dr Bajaj’s research has been funded through the NIH, US Veterans Affairs and American College of Gastroenterology. His work has been published in Gastroenterology, Hepatology, the American Journal of Gastroenterology.
of Gastroenterology and Liver Transplantation, among others. Dr Bajaj is an Associate Editor for the American Journal of Gastroenterology and Journal of Clinical and Experimental Hepatology and is on the editorial board for Alimentary Pharmacology and Therapeutics and Liver International. Dr. Bajaj frequently presents his work on hepatic encephalopathy and chronic liver disease nationally and internationally at meetings grand rounds, and symposia and is a member of the Hepatic Encephalopathy Guidelines Writing Committee. He is also a member of several medical associations, including the American Gastroenterological Association, the American Association for the Study of Liver Disease, and the American College of Gastroenterology. Dr Bajaj is the Chairperson of the Acute-on-Chronic Liver failure Special Interest Group at the American Association for the Study of Liver Disease and for the North American Consortium for Study of End-stage Liver Disease.

Dr. Arthur Cooper
Dr. Cooper is Professor of Biochemistry and Molecular Biology at New York Medical College and Adjunct Professor of Biochemistry in Neuroscience at the Weill Medical College of Cornell University. His research interests include pyridoxal 5'-phosphate-enzymes, enzymatic mechanisms, bioactivation mechanisms, neurochemistry, neurodegenerative diseases, chemoprevention, and 1-C, nitrogen, sulfur and selenium biochemistry.

Sriravasan Dasarathy
My talk will be on Skeletal muscle and ammonia disposal more than just metabolism. Staff transplant hepatologist at the Cleveland Clinic with specific interest in mechanisms of skeletal muscle loss in liver disease. Our laboratory focuses specifically on transcriptional signaling pathways that regulate skeletal muscle protein synthesis and autophagy during hyperammonemia of cirrhosis using metabolic tracer studies and molecular pathway analyses.

Dr. Nathan Davies
Nathan Davies is a Senior Lecturer at the Institute for Liver and Digestive Health in University College London’s Division of Medicine. His academic interests relate the processes related to inflammation and their effects on the body’s systems during liver disease processes. Current projects include testing whether modulating gut bacteria reduces systemic inflammation in liver patients has a measurable health benefit.

Nicolaas E Deutz, MD, PhD
Title of Presentation: Targeted fluxomics in humans to measure the synthesis and breakdown of biological molecules. Nicolaas E Deutz, MD, PhD is active in the field of Clinical Nutrition and Metabolism, presently improving skeletal muscle. At Texas A&M University, he is a tenured and endowed Professor and director and founder of the 6,000 sqft Center for Translational Research in Aging and Longevity Center (CTRAL; http://ctral.org).

Radha Krishan Dhiman, MD, DM, FACC, FAMS
Professor, Department of Hepatology, Postgraduate Institute of Medical Education and Research, Chandigarh (PGIMER), India

SPEAKERS

Dr William Bernal
Dr William Bernal is currently Consultant and Reader in Liver Intensive Care Medicine in the Liver Intensive Therapy Unit at the Institute of Liver Studies at King’s College Hospital in London UK. He trained in General Medicine at the Royal London and St Bartholomew’s Hospital in London and in Hepatology and Intensive Care Medicine at St Thomas and King’s College Hospital. He first worked on Liver Intensive Therapy Unit at King’s in 1995 and was appointed as a Consultant in 2002. Annually, more than 1200 patients with liver disease are admitted to this specialist critical care unit which also supports one of Europe’s largest liver transplantation programs. The unit has a special interest in acute liver failure and has for many years led in the development and application of innovative therapies for this condition. Dr Bernal’s research interests include the pathogenesis of encephalopathy and multiple organ failure in acute and acute-on-chronic liver failure and the use and outcome of liver transplantation. He has published widely in this field and his current research projects include the application of novel techniques to prognostic assessment and the impact of body composition changes on survival in patients with liver disease.

Prof Roger Butterworth
PhD (University of London, UK) in 1989, DSc (University of London, UK) in 1996. Until retirement in 2013, Dr Butterworth held the following appointments: Full Professor, Dept of Medicine, University of Montreal, Adjunct Professor, Division of Experimental Medicine, McGill University and Director, Neuroscience Research Unit, St-Luc Hospital (CHUM), President, International Society of Neurochemistry (2006), President, International Society for Hepatic Encephalopathy and Nitrogen Metabolism (ISHEN) (2009), Editor-in-Chief, Metabolic Brain Disease (1994-1998) Editor-in-Chief, Neurochemistry International (1998-2010), Member, Editorial Board, Hepatology, Journal of Hepatology, Gold Medalist, Canadian Association for the Study of the Liver (2003), Founding Member, Society of Neuroscientists of Africa (SONA), Order of Senegal for Neuroscience teaching and Research in Africa (1998). 725 publications on basic and clinical research in Metabolic Disorders of the CNS including Wernicke-Korsakoff Syndrome, Urea Cycle Enzymopathies and Hepatic Encephalopathy.

Nicolaas E Deutz, MD, PhD
Title of Presentation: Targeted fluxomics in humans to measure the synthesis and breakdown of biological molecules.

Dr Radha Krishan Dhiman
MD, DM, FACC, FAMS
Professor, Department of Hepatology, Postgraduate Institute of Medical Education and Research, Chandigarh (PGIMER), India

MAJOR HONORS
1. Dr BC Roy National Award; This is highest award in the field of Medicine in India; Received from Honorable President of India Smt Pratibha DeviSingh Patil on July 2, 2008.
3. Harold O Conn award by American Association for the study of Liver (AASLD) at the Annual meeting held in November, Boston, USA.

RESEARCH INTEREST: Hepatic encephalopathy and portal hypertension.

Dr Daniel Forton
Dr Daniel Forton BSc, PhD, MBBS, FRCP is a Consultant Hepatologist and Reader at St. George’s University of London, UK.

Vicente Felipo
Vicente Felipo obtained his Ph.D degree in Biochemistry from the University of Valencia in 1983. In 1990 he created the Laboratory of Neurobiology of Centro de Investigacion Principe Felipe, Valencia, Spain. He is Director of the Laboratory and of the Program on Neurologic Impairment of the Center.

Prof Nicolaas E Deutz
Nicolaas E Deutz, MD, PhD is active in the field of Clinical Nutrition and Metabolism, presently improving skeletal muscle. At Texas A&M University, he is a tenured and endowed Professor and director and founder of the 6,000 sqft Center for Translational Research in Aging and Longevity Center (CTRAL; http://ctral.org).

Dr. Arthur Cooper
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Prof Alfiaz A. Forton
Alfiaz A. Forton is Professor of Medicine in the Norwich Medical School (UEA). He was previously at UCL/UCLH and St. Mark’s Hospital in London. He has been Education Director for the European Society for Clinical Nutrition (ESPEN), Secretary of the British Society of Gastroenterology, and Chairman of the British Association for Parenteral and Enteral Nutrition. His interests lie mainly in intestinal failure and IBD. His CV has over 220 original papers, with an h score of 49, together with many review articles and contributions to guidelines.

Dr. Daniel Forton
Dr Daniel Forton BSc, PhD, MBBS, FRCP is a Consultant Hepatologist and Reader at St. George’s University of London, UK.

Dr. Arthur Cooper
Dr. Cooper is Professor of Biochemistry and Molecular Biology at New York Medical College and Adjunct Professor of Biochemistry in Neuroscience at the Weill Medical College of Cornell University. His research interests include pyridoxal 5'-phosphate-enzymes, enzymatic mechanisms, bioactivation mechanisms, neurochemistry, neurodegenerative diseases, chemoprevention, and 1-C, nitrogen, sulfur and selenium biochemistry.

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Nicolaas E Deutz, MD, PhD
Title of Presentation: Targeted fluxomics in humans to measure the synthesis and breakdown of biological molecules.

Dr Radha Krishan Dhiman
MD, DM, FACC, FAMS
Professor, Department of Hepatology, Postgraduate Institute of Medical Education and Research, Chandigarh (PGIMER), India

OFFICE BEAVER
3. Governor of American college Gastroenterology (ACG) for India.
Dr. Rita Garcia-Martinez
Dr. Rita Garcia-Martinez earned her medical specialty in Internal Medicine-Hepatology in Vall d’Hebron. During her predoctoral training she focuses on hepatic encephalopathy and the long-term consequences after successful liver transplant under the supervision of Prof. Juan Cordoba. After her postdoctoral fellowship in UCL-London conducting a translational program in liver failure she is back in Spain working in liver failure and its complications.

Dr. Oec. Troph. Boris Görg
Boris Görg studied nutritional sciences and graduated with diploma at the University of Born in Germany. He completed his doctorate dealing with molecular mechanisms involved in the pathophysiology of hepatic encephalopathy at the Department of Gastroenterology, Hepatology and Infectiology of the University Clinic of Düsseldorf where he is currently working as a scientific research associate. His scientific research focuses on the role of osmotic and oxidative/nitrosative stress in the pathophysiology of hepatic encephalopathy.

Professor Elaine Holmes
Professor Holmes (PhD) is a Professor of Chemical Biology at Imperial College London and Head of Division of Computational and Systems Medicine. Her main research area focuses on applying metabolic profiling and computational modelling of biofluids and tissues to understand pathological and physiological processes. She has a broad background in metabolic chemistry, with specific expertise in spectroscopy and in chemometric modeling of spectral data. She began her research career investigating molecular mechanisms of toxicology using spectroscopic methods and then broadened the scope to research clinical pathologies in a range of clinical fields. Prof Holmes has several research projects investigating the consequences of modification of the gut microbiota and has particular interest in the gut-brain axis. Her research involves both the development and application of spectroscopic and chemometric methods, and in particular the fusion of metagenomic and metabonomic data to provide a readout of the functionality of the microbiome. She has also been involved in development of methods for improving the extraction of latent biomarker information from spectral data. She holds visiting Professorships with a number of universities including CEU Madrid and the Chinese Academy of Sciences in Wuhan.

Tarek Hassanein
Tarek Hassanein MD, FACCP, FACC, AGAF
Dr. Tarek Hassanein is board certified in Gastroenterology and Hepatology and is a Professor of Medicine at the UCSD School of Medicine. A leading expert in liver diseases, Dr. Hassanein is renowned for management of viral hepatitis, fatty liver, cirrhosis, liver cancer, and pre/post liver transplant care. Prior to joining SCLC, Dr. Hassanein was the Director of the UCSD Liver Center and Medical Director of Liver Transplantation from 1997 to 2009. Dr. Hassanein earned his medical degree from Alexandria University in Egypt, and completed Residency in Internal Medicine at Wayne State University in Detroit, Michigan. He completed a Research and Clinical Fellowship in Gastroenterology, Hepatology, and Transplantation at the University of Pittsburgh in 1994.

Professor Dr. Dieter Häussinger
Professor Dr. Dieter Häussinger was born 22.06.1951 in Nördlingen/Bavaria. Full Professor of Internal Medicine and Director of the Clinic for Gastroenterology, Hepatology and Infectious Diseases at the Heinrich-Heine-University Düsseldorf. Director of the Center of Liver and Infectious Disease and of the W. Hirsch Institute for Tropical Medicine at Adama University (Ethiopia). Dean of the Medical Faculty (1998-2002). Member of the Medical School and Creditation Board. Since 2008, he has been a member of the faculty of the Department of Nuclear Medicine & PET Centre, University of Copenhagen, DK-2100 Copenhagen, Denmark. Special interest in the gut-brain axis. Her research involves both the development and application of spectroscopic and chemometric methods, and in particular the fusion of metagenomic and metabonomic data to provide a readout of the functionality of the microbiome. She has also been involved in development of methods for improving the extraction of latent biomarker information from spectral data. She holds visiting Professorships with a number of universities including CEU Madrid and the Chinese Academy of Sciences in Wuhan.

Gerald Kircheis
Date of Birth: 13.09.1957 in Teutscnhenthal, Germany Degrees, Diplomas, past and present positions

Professor Rajiv Jalan
Professor Rajiv Jalan is Professor of Hepatology at the UCL Medical School. He has a strong clinical academic interest in translational research in the area of liver failure. He has been closely involved in the discovery of ornithine phenylacetate; a new treatment for hepatic encephalopathy. His work provided the seminal observation of the role of hypothermia in patients with uncontrolled intracranial hypertension, an intervention that has become routine in many units around the world. His research has focused on acute-on-chronic liver failure (ACLF), a syndrome which he identified as a new clinical entity. He is currently the President of ISHEN and Editor-in-Chief of the Journal of Hepatology.

Radhakrishnan Jayakumar
Dr. Jayakumar is a Research Associate Professor of Neuroscience at the Miami VA Hospital. He is currently examining the role of astrocytes in acute/chronic hepatic encephalopathy. His research interests include the role of blood-brain barrier in the development of cytotoxic brain edema, mechanisms of BBB integrity, and cell-cell interaction in HE.

Wim Laleman
MD
Dr. Laleman earned his M.D. from Catholic University of Leuven in 2000 where he completed his internal medicine residency, gastroenterology-hepatology fellowship and advanced training in endoscopic retrograde cholangiopancreatography (ERCP) and endoscopic ultrasound (EUS). In 2006, he obtained his Ph.D. on “The role of hepatic stellate cells and vascular mediators in the pathophysiology of cirrhotic portal hypertension”. Since 2008, he has been a member of the faculty of the Department of Liver and Biliarypancreatic disorders at the Leuven University Hospitals. In addition to continuous clinical and teaching assignments, he has a strong research interest, both clinically and basically, on complications of cirrhosis focusing on portal hypertension (variceal hemorhage, ascites, ...) and liver insufficiency (hepatic encephalopathy, bacterial translocation, ...) leading to numerous publications. In this capacity, he was granted a Senior Clinical Investigatorship by the Research Foundation-Flanders since 2010 and was awarded the Prize George Broheé in 2009.
Dr. Lateman is currently Secretary of the Belgian Association for the Study of the Liver (BASL). He is member of European Association for the Study of the Liver (EASL) and the European Society for Gastrointestinal Endoscopy (ESGE) and serves as manuscript reviewer for several medical journals, including, amongst others, Endoscopy, the Journal of Hepatology, Hepatology, the European Journal of Gastroenterology and Hepatology and Liver International.

Andrew Langford
Chief Executive, British Liver Trust
Andrew Langford joined the British Liver Trust in August 2011. With a wealth of experience, Andrew has worked in Chief Executive and trustee roles for an umbrella dermatology charity, voluntary sector hospices and HIV, cancer and children’s charities. Andrew has a distinguished reputation in the charity sector, being chosen to Chair the Parliamentary Inquiry into Skin Cancer and also developed an internationally recognised centre of excellence in the hospice care of people with HIV related encephalopathy. Andrew’s most recent role as the Chief Executive of the Skin Care Campaign gave him the opportunity to raise the profile of skin conditions in the UK, ensuring the Campaign was a fundamental player in the dermatology health community and policy areas.

With over 30 published papers with the BMJ, HSJ and other professional journals, Andrew has embraced his role in improving care in various health sectors, such as dermatology, HIV, palliative care and dementia.

Early on in his career, Andrew trained as a registered general nurse before returning to academia to undertake a degree in History at Oxford University and then continued his education with a Masters degree in Political History at Huddersfield University. Andrew is a keen rugby fan, and is a reluctantly retired rugby player, although still a keen fan.

Fin Stolze Larsen
Consultant at Dept. Hepatology, Rigshospitalet, University Hospital of Copenhagen. Responsible for the liver ICU, and Lab., research activities. Doctor of Medical Science (DMSc) and Ph.D. Author of a number of scientific papers, mainly with emphasis on metabolic encephalopathy, cerebral oedema, systemic haemodynamics, liver support devices and liver regeneration. Principle Advisor for several PhD- and DMSc-thesis (DMSc.) from the Dept. Hepatology as well as member/chair of the evaluation committee of numerous PhD. theses. Steering member of the acute liver failure group, the CLIF consortia and reviewer for several international journals, universities, scientific organizations and foreign governmental research institutions.

Dr Alistair Lee
Dr Alistair Lee has been a consultant in Critical Care and Transplant Anaesthesia in Edinburgh, UK since 1991 with a particular interest in acute liver failure. The Edinburgh group produced some of the earliest studies on the use of induced hypothermia in the management of raised intracranial pressure in acute liver failure in the late 1990s. Additional studies examined cerebral autoregulation in this condition, the effects of liver reperfusion on cerebral haemodynamics, and the use of hypothermia during orthotopic liver transplantation. Dr. Lee has contributed to the development of a porcine model of acute liver failure secondary to paracetamol poisoning, and has experience in the use of albumin dialysis and bioartificial extracorporeal liver support systems in acute liver failure. He has conducted earlier studies on systemic haemodynamic changes in liver transplant recipients using different methods of reperfusion, and acute liver failure patients receiving N-acetylcysteine. Dr. Lee has examined the use of near infrared spectroscopy as a non-invasive method to measure intracranial pressure, and more recently the use of optic nerve sheath ultrasound measurements as a surrogate marker of intracranial pressure. He is currently Secretary / Treasurer of LICAGE (Liver Intensive Care Group of Europe) and has spoken extensively on a range of topics associated with acute liver failure and liver transplant anaesthesia.

Julian Marchesi
Julian Marchesi graduated from Cardiff University with a PhD in biochemistry (1992) and became interested in gastrointestinal microorganisms (i.e. gut microbiota). His work uses a variety of “omic” approaches such as metagenomics, metatranscriptomics, metabolomics and molecular ecology to investigate the human gut microbiome. Over his 25 year career he has authored over 75 peer-reviewed publications and is an editor/ senior editor for ISME Journal, FEMS Microbiology Ecolog and BMC genomics and is a current member of the editorial boards of Journal of Medical Microbiology and Microbiome and past member of the Journal Microbiology Methods, Current Issues in Molecular Biology, and Molecular Biology Today.

Prof Manuela Merli
Professor Merli is a consultant hepatologist and gastroenterologist at the Department of Gastroenterology II Polyclinic Umberto I, University La Sapienza of Rome. She currently teaches internal medicine and gastroenterology in the schools of medicine and nursing. Prof. Merli graduated in medicine in 1980 and is an author of over 240 scientific publications. Her research activities include, among other things, chronic hepatitis, liver cirrhosis, and liver transplantation. Prof. Merli has particular expertise in nutrition in the context of liver disease and has published extensively in this area.

Dr Sara Montagnese (MD, PHD)
Dr Sara Montagnese (MD, PhD) is a Reader in Medicine and Honorary Consultant Physician at the University of Padova, Italy. Her research interests lay in metabolic encephalopathies, particularly hepatic encephalopathy, sleep and circadian rhythms, on which she has published extensively.

Dr Rajeshwar Mookerjee
Dr Mookerjee is a UCL senior Lecturer and a consultant in hepatology at the Royal Free Hospital, London. His clinical and research interests focus on the complications of chronic liver disease including vascular dysfunction in cirrhosis and its intervention, and the evolution of acute-on-chronic liver failure. Integral to this process is the understanding of the role of infection and inflammation in provoking organ injury. This is on-going research, coupled with the development of early prognostic markers to predict outcome in liver failure. Studies of relevant biomarkers are also being used to help inform the design of new interventions in liver disease. The focus of the translational research program is exploring hypotheses in models of liver disease to elucidate the pathophysiologically deranged observations in patients.

Marsha Y Morgan
Qualified in medicine with distinction and undertook her early clinical training in Manchester and London. Undertook specialist training in Gastroenterology in London and then joined Professor Dame Sheila Sherlock in the Department of Medicine at the Royal Free Hospital School of Medicine, initially as a Research Fellow but subsequently as Lecturer and then Senior Lecturer in Hepatology within the same Department. Following the merger with University College London, she was promoted to Reader in Medicine and subsequently to the position of Principal Research Fellow. Her main research interest is in alcohol misuse and alcohol-related liver disease but she also has considerable research interests in hepatic encephalopathy and nutrition in liver disease. Her interest in alcohol misuse and alcohol-related liver disease has resulted in appointments to the World Health Organisation Expert Committee on Drug Dependence and Alcohol Problems; the Ministry of Transport Medical Advisory Panel on Alcohol, Drugs and Substance Misuse and Driving and the Royal College of Physicians and the Royal College of Psychiatrists Working Parties on Alcohol and the Institute for Alcohol Studies. In the last three years she has been involved in the development of five major NICE documents. She is currently actively engaged in research into the genetics of alcohol-related cirrhosis.

Her interests in hepatic encephalopathy have resulted in her appointment to the Executive Board of the International Society for Hepatic Encephalopathy and Nitrogen Metabolism (ISHEN). Her current research interests are in the utility of neurophysiological variables for the diagnosis of hepatic encephalopathy and the efficacy of various treatment options. Her interest in nutritional aspects resulted in her playing a major role in the development of the ISHEN guidelines for the nutritional management of hepatic encephalopathy. She has recently validated two new methods for assessing nutritional status in patients with cirrhosis which are likely to become the gold standards for use in this population. Her current research interest is in the management of malnutrition in patients with chronic liver disease.

Prof Kevin Mullen
Professor Kevin Mullen graduated from medical school in Dublin (UCD) and trained in McMaster University in Internal Medicine. His Gastroenterology and Hepatology training were obtained in Case Western Reserve University and the National Institutes of Health respectively. He has been on staff in Metrohealth Medical Center since 1986 and Became Professor of Medicine in 1999.
Dr. Jude A Oben BM.BCh(Oxon), PhD, FRCP
(Lond & Edin)
After medicine at Oxford (St John’s College), with a stint at Stanford as a research fellow in Immunology, a foray into ophthalmology was followed by critical care at St Thomas’ Hospital and a decision on Hepatology as a specialty. Post-doctoral fellowship was at Johns Hopkins University, mentored by Professor Anna Mae Diehl leading to a Wellcome Intermediate Fellowship. Dr Oben’s productive research Group focuses on mechanisms of non-alcoholic fatty liver disease and autoimmunity, with a particular emphasis on the autonomic nervous system regulation of liver repair.

Marc Oria
PhD
I finished my degree in Biology at the University of Barcelona (Spain) in February 2003, and followed this with PhD studies in the group of metabolomics and elderly encephalopathies, led by Professor Joan Cordoba, which was awarded in March 2011. My research interests during the PhD were focused on the study of the neuronal function in different liver failure animal models. During this period, I also obtained a DEA in Neuroscience (specialized post-graduate research course in Biomedicine, mandatory to obtain a PhD) from the University Autonoma of Barcelona. From September 2003 to January 2004 I had the opportunity to work in close collaboration with the Gastroenterology Group of the Germans Trias y Pujol Hospital (Badalona, Spain), under the supervision of Dr Ramón Bartolí and Dr. Josep Mañé. During this period I gained expertise in the induction of cirrhosis in animal models and worked with IL-10 knockout mice in models of inflammatory intestinal diseases. In February 2004, I worked in the neuroscience laboratory in Hospital Saint-Luc (Montreal, Canada), led by Dr. Roger Buttersworth, where he learned the portacaval anastomosis surgical technique in order to develop the model of hepatic encephalopathy in animal models. In 2008 I did collaboration with Pr. Mihajlov and Dr. Erceg in the Principe Felipe Research Center (Valencia) assessing the function of the motor tract in rats after spinal cord injury.

In July 2010, I visited the Cellular Biology and genetics department in the Alcalá de Henares University (Alcalá de Henares, Spain) with the objective of assessing the process of injury to the motor tract in experimental animals. This visit initiated a series of collaborations with Dr. Guillelmo Bodega, a neuropathologist who later visited the laboratory of Dr Cordoba in Barcelona. In September 2012, I was awarded a Damme Sheila Sherlock EASL Post-Doctoral Fellowship to develop a new project in the University College of London (UCL) with Prof. Rajiv Jalan. During this post-doc experience I developed new skills in molecular biology and working in the senescence pathways activated in the brain from liver disease animal models.

In June 2012, I established a new Collaboration with Dr. Ercog and Dr. Moreno in the Principe Felipe Research Center (Valencia) assessing the function of the motor tract in rats after spinal cord injury. During this process I established relationships with several scientists around the world and I published in top journals with high impact factor. In 2012 I was awarded with the Post-Doctoral EASL, Dame Sheila Sherlock Grant to study the Neurodegenerative process present in Liver failure animal models of liver disease in the Liver Failure group, Royal Free Hospital, UCL (London) lead by Professor Rajiv Jalan. In 2014 I started a new research line in the Translational Research in Fetal Surgery for Congenital Malformations Center for Fetal, Cellular and Molecular Therapy at the Cincinnati Children’s Hospital Medical Center (CCHMC) studying the effect on the central nervous system by spina bifida and possible therapies.

Peter Ott, Dr.MSc. MD
Assistant Professor
Head of Medical Department V. Hepatology and Gastroenterology
Aarhus University Hospital. Denmark
Peter Ott graduated from the Medical Faculty in Copenhagen. He received his training in Hepatology at Department A at Copenhagen University Hospital which was then headed by Professor Niels Tygstrup. Peter Ott specialized as a hepatologist in 1996 and served as a consultant at Copenhagen University Hospital until he became Head of Department V at Aarhus University Hospital in 2004. This has a highly specialized hepatological profile in Denmark and a strong research activity. He is the academic leadership of professor Hendrik Vilstrup, the lab now involving 18 PhD students and a production of around 50 peer reviewed papers a year. Peter Ott has published more than 135 papers including reviews, textbook chapters, and research papers. His longstanding interest in hepatic encephalopathy focus on the consequences of ammonia metabolism and clinical course in acute and chronic liver failure.

Oliviero Riggio
Oliviero Riggio, born in Roma on 14/11/1952, married, 3 children, is Associate Professor of Gastroenterology at “Sapienza” University of Rome. Oliviero Riggio’s main research topic is the complications of liver failure. In this field, by using both clinical methodologies and experimental models, he studied the pathophysiology and treatment of hepatic encephalopathy, focal liver diseases and alcoholic hepatitis. He investigated the mechanisms of alcoholic liver disease and other substrates in isolated tissues “in vitro”, the euglycemic clamp technique and indirect calorimetry. He developed and used experimental models of liver failure such as the portalacaval shunt and the carbon tetrachloride-induced cirrhosis in the rat. He carried out randomized controlled trials on branched chain amino acids, lactitol and zinc as new drugs in the treatment of hepatic encephalopathy, and on TIPS in the prevention and treatment of the complications of portal hypertension. He published more than 200 papers. His H-index is 31 (according to Web of Knowledge).

Don C. Rockey, M.D.
Don C. Rockey received his bachelor’s degree in biology (summa cum laude) from Virginia Polytechnic Institute and State University, and he earned his M.D. from the Medical College of Virginia. Dr. Rockey completed his internship, residency and fellowships in Gastroenterology at the University of California San Francisco. In 1997 Dr. Rockey joined the faculty at Duke University Medical Center as Chief of Hepatology and Director of the DUMC Liver Center in North Carolina. In 2005 he moved to Texas, to head the Division of Digestive and Liver Diseases at UTSW. In 2012 Dr. Rockey joined the Medical University of South Carolina as Chair for the Department of Medicine.

Christopher Rose
Christopher Rose received his PhD (Biomedical Sciences) from the Université de Montréal in 2000 under the supervision of Prof. Butterworth. He continued his research interests in Europe by pursuing post-doctoral studies in Germany (Prof. Kettenmann, Berlin), Spain (Prof. Felipe, Valencia and Prof. Cordoba, Barcelona) and Norway (Dr. Ytrebo, Tromso). In doing so, he was the recipient of 3 prestigious post-doctoral fellowships from the European and the Alexander von Humboldt Foundation (Germany), CIHR (Canada) and the European Association for the Study of the Liver (Europe). He is currently Associate professor in the Department of Medicine at the Université de Montréal and his laboratory, Hepato-Neuro, has been established at the CRCHUM since 2006. Dr. Rose has published over 50 articles, many in high-impact journals such as New England Journal of Medicine, Gastroenterology, Hepatology and Journal of Hepatology. His research interests lie within the area of hepatic encephalopathy, neuroplasticity, the alterations of glucose, lipid and protein metabolism, the alterations in nutritional state and the therapy of portal hypertension. For the above purposes he developed and utilized different laboratory neurobiology and clinical methodologies and experimental models, he studied the pathophysiology and treatment of hepatic encephalopathy, focal liver diseases and alcoholic hepatitis. He investigated the mechanisms of alcoholic liver disease and other substrates in isolated tissues “in vitro”, the euglycemic clamp technique and indirect calorimetry. He developed and used experimental models of liver failure such as the portalacaval shunt and the carbon tetrachloride-induced cirrhosis in the rat. He carried out randomized controlled trials on branched chain amino acids, lactitol and zinc as new drugs in the treatment of hepatic encephalopathy, and on TIPS in the prevention and treatment of the complications of portal hypertension. He published more than 200 papers. His H-index is 31 (according to Web of Knowledge).
**SPEAKERS**

**Professor Freimut Schliess**
Professor Freimut Schliess is a biochemist with longstanding contributions in the fields of Experimental Hepatology and Molecular Medicine. He received his scientific training in the laboratory of Prof. Dr. Dieter Häussinger (Heinrich-Heine-University, Düsseldorf, Germany). Major scientific contributions include the discovery of hepatic insulin resistance mechanisms and of key cerebral pathways in Hepatic Encephalopathy. Today he heads the Scientific Affairs department at Profil Institute of Metabolic Research (www.profil.com, Neuss, Germany), a privately owned research institute contributing to early phase clinical development of drugs and medical devices for people with diabetes. Freimut Schliess has awarded the Heinz Kalk prize for scientific achievements in hepatobiliary transport physiology and metabolism. Publications: http://www.ncbi.nlm.nih.gov/pubmed?term=Schliess

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**Peter Seardon**
Peter Seardon is the Reynolds Professor of Engineering and Director of the Institute for Nanobiotechnology at Johns Hopkins University. He received his PhD from the University of Manchester in England and is a fellow of the American Physical Society and the American Association for the Advancement of Science.

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**Prof Barjesh C Sharma**
Prof. Barjesh C Sharma is a professor of gastroenterology at Govind Ballabh Pant Hospital, New Delhi. He has published over 180 papers in the area of gastroenterology and liver diseases and has particular expertise in hepatic encephalopathy and studies evaluating treatments for this condition. Prof Sharma is currently the Secretary General-cum-Treasurer for the Asian Pacific Association for the Study of the Liver (APASL), and also serves as chairman of the Ethics and Guidelines committee.

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**Debbie Shawcross BSc, MB BS (distinc); PhD; FRCP**
Debbie Shawcross is a Clinician Scientist based at the Institute of Liver Studies, King’s College Hospital. She held a HEFCE Clinical Senior Fellowship between January 2008 and 2013 and works as a Consultant Hepatologist on the King’s Liver Unit with a specialist interest in hepatic encephalopathy. She is the lead for Education and Training in Hepatology within King’s Health Partners, the Academic Health Sciences Centre and is the Training Programme Director for Gastroenterology and Hepatology Specialist Training in South Thames. The aims of her ongoing research programme are to characterise the molecular mechanisms underlying the predisposition to infection in liver failure focusing specifically on neutrophil dysfunction and hepatic encephalopathy.

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**Michael Serensen**
Michael is an M.D. and currently in training as a hepatologist. He has a Ph.D. and is specialized in functional investgation of liver and other organs affected by diseases of the liver. His work within the field of hepatic encephalopathy has focused on metabolic changes in vivo.

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**Dr Mark Swain**
Dr. Mark Swain is currently Professor of Medicine and a hepatologist (Liver Specialist) at the University of Calgary, where he is also Head of the Division of Gastroenterology and Hepatology. He graduated from an MD-MSc (Pharmacology) combined program at Queen’s University, Kingston in 1985, and then completed his Internal Medicine training at the University of Toronto (1985-88) and his Gastroenterology training at McMaster University in Hamilton (1988-90). He then undertook additional training as a Hepatology Medical Staff Fellow in the Liver Unit at the National Institutes of Health, Bethesda Maryland, USA (1990-93). Dr. Swain is currently a clinician-scientist at the University of Calgary where he is funded by the Canadian Institutes for Health Research for basic laboratory-based bench research in 2 main areas: (i) mechanisms underlying the development of sickness behaviors (ie. symptoms) in the context of liver disease, and (ii) the innate immune response and hepatic inflammation. His clinical research is focused in the areas of viral hepatitis and autoimmune liver disease. Dr. Swain has published numerous peer-reviewed papers and book chapters and has served on the Editorial Boards for a number of scientific journals including Gut, American Journal of Physiology and Clinical Sciences. He has won many awards for teaching and research, including the University of Calgary Watanabe Distinguished Achievement Award for Overall Excellence. He currently holds the Cal Werzel Family Foundation Chair in Hepatology, and is Head of the Translational Research Core for the Snyder Institute for Chronic Diseases at the University of Calgary.
Simon Taylor-Robinson
Professor Simon Taylor-Robinson joined Imperial College London in 1997, becoming Professor of Translational Medicine in 2007. He was the Dean of the Faculty of Medicine at Imperial College from September 2010-September 2013. He is a career hepatologist with clinical appointments as a consultant at Imperial College Healthcare Trust in London (St Mary’s and Hammersmith Hospitals). Over the past 25 years, he has investigated pathogenic mechanisms in chronic liver disease using a combination of imaging techniques, NMR spectroscopy and mass spectroscopy. He has over 210 publications currently. Professor Taylor-Robinson has strong connections with Africa, having forged research partnerships through European Framework 7 with clinical and research groups in Nigeria, Senegal and Gambia. He has visited many African countries in a research and clinical capacity, including South Sudan, Sudan and Kenya. In June 1999, he received a MRC Career Establishment grant for liver fibrosis. This generated industrial sponsorship from Pfizer and GSK. He holds grants from MRC (one is joint with Newcastle, Cambridge and Birmingham University on PBC), NIHR and Wellcome Trust. He is clinically responsible for patients with liver disease at St. Mary’s Hospital (Imperial College Healthcare Trust) and is currently Director of the Imperial Clinical Research Facility, which has access to the largest cohort of viral hepatitis patients for study in Europe, in addition to well characterised patients with fatty liver disease and PBC. He was awarded the Sir Francis Avery Jones Gold Medal by the British Society of Gastroenterology in 1999 and Linacre Lectureship of the Royal College of Physicians of London in September 2000.

Dr. Alexander S. Thrane
Dr. Alexander S. Thrane received his MBChB (Hons.) from the University of Leicester, United Kingdom, in 2008. After completing foundation year training at Haukeland University Hospital in Bergen, Norway, he went on to study astrocyte physiology and pathophysiology as a PhD fellow. His work examined astroglial water and ion regulation in the context of brain disorders like oedema, migraine, hepatic encephalopathy, hyperammonaemia, as well as during general anaesthesia. The research was sponsored by a Fulbright scholarship, and completed partly at the University of Rochester, NY with Prof. Makken Nedergaard and partly the University of Oslo with Prof. Erlend Nagelhus. Dr. Thrane received his PhD in two-photon imaging and neurophysiology in 2013, and is currently an ophthalmology resident and postdoctoral fellow at Haukeland University Hospital in Bergen, Norway. His more recent research interests include next-generation genetic analyses and optical imaging in hereditary retinal diseases and Müller glial cell pathophysiology.

Javier Vaquero
Javier Vaquero MD, PhD, performed research on hepatic encephalopathy in the laboratories of Dr. AT Blei (Chicago, 2000-2003) and Dr. RF Butterworth (Montreal, 2003-2008), and on liver regeneration in the laboratory of Dr. N Fausto (Seattle, 2008-2011). He currently leads a Hepatology research laboratory at Hospital Gregorio Marañón (Madrid, Spain).

Hendrik Vilstrop
MD DSc FRCP FEBGH
Professor of Medicine and Hepatology and Department Chair, Department of Hepatology and Gastroenterology, Aarhus University Hospital Denmark.

Prof Karin Weissenborn
Prof Weissenborn has been based for many years at Hannover Medical School in Germany and is currently is Associate Professor of Neurology. She has major research interests in Metabolic encephalopathies, HCV-encephalopathy and strokes.

Professor Roger Williams
CBE, MD, FRCP, FRCS, FRCPCE, FRACP, FMedSci, FRCPI (Hon), FACP (Hon)
Director, Institute of Hepatology, London and Foundation for Liver Research
Roger Williams is Director of the Institute of Hepatology, London and of the Foundation for Liver Research. Before that, he had established over a period of 30 years, the world renowned Institute of Liver Studies at King’s College Hospital. He is a Fellow of the Academy of Medical Sciences and is the recipient of numerous honorary fellowships, medals and prizes including the American Society of Transplantation Senior Achievement Award in 2004, a Hans Popper Lifetime Achievement Award in 2008, the Distinguished Service Award of the International Liver Transplant Society in 2011, and in 2013 the Distinguished Achievement Award of the American Association for the Study of Liver Disease. His main clinical and research interests are in acute liver failure, liver transplantation, complications of cirrhosis and management of viral hepatits.
ISHEN

Gala Dinner
Northcote House

Medieval Themed Night

12th September 2014
7.30pm
Smart or medieval dress code
CONFERENCE LOCATION
Sunningdale Park, Larch Ave, Ascot, Berkshire SL5 0QE

REGISTRATION DESK OPENING HOURS:
Wednesday 10th September 2014 10.00 am – 7.35 pm
Thursday 11th September 2014 7.30 am – 6.30 pm
Friday 12th September 2014  8.00 am – 6.30 pm
Saturday 13th September 2014  7.30 am – 11.00 am

EXHIBITION OPENING TIMES:
Wednesday 10th September 2014 12.30 pm - 7.35 pm
Thursday 11th September 2014  8.00 am – 6.30 pm
Friday 12th September 2014  8.00 am – 6.00 pm
Saturday 13th September 2014  7.30 am – 10.45 am

REFRESHMENT BREAKS AND LUNCHES
All lunches will be held at the “Steam Bake and Grill” restaurant just outside the conference centre.
Lunches will be served on each day to all delegates wearing an official ISHEN name badge.
Refreshments will be served in the concourse area in the conference centre.
Where possible, products containing modified soya and maize have been avoided but some products may contain ingredients produced from genetically modified maize.
Some of the menu items may contain nuts, seeds and other allergens and there may be a risk that traces of these could be in any other dish or food served at the conference. Please inform the registration desk on arrival of any special dietary requirements.
Water will be available from fridges at all times during the symposium - please help yourself.

OFFICIAL LANGUAGE
The official language of the conference is English.

SOCIAL PROGRAMME
Leaflets detailing local attractions and theatre productions can be found at the Hotel reception desk. If you require information on local restaurants please ask at the Hotel reception desk.

QUERIES
If you have any other queries or issues relating to your attendance at the conference, please contact the onsite Organisers Office +44 (0) 1344 634118. Following the event, all queries should be directed to the ISHEN Administration office.

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Website: www.ishen.moonfruit.com

GENERAL INFORMATION

CLIMATE
The average maximum temperature in Ascot during September is 15ºC with a chance of rain.

ELECTRICITY
The electrical current in the United Kingdom is 240 volts with 3 Pin Plugs. Most hotels provide 110 volt outlets for shavers.

INSURANCE
The conference organisers cannot accept liability for personal injuries sustained, or for loss (or damage to) property belonging to conference participants (or their accompanying persons), either during or as a result of the conference.

MOBILE TELEPHONES
Please ensure that all mobile telephones are switched off during all sessions.

SMOKING POLICY
The Organisers wish to stress that this will be a ‘No Smoking’ conference. Smoking in public places Around Ascot, such as restaurants and on public transport, is not permitted.

POSTER VIEWING AND DISCUSSION
Poster viewing will be in the Whitley/Mandela room.

Oral Presentations will also be made throughout the conference programme.
Please see programme for presenter timings.

CPD
ISHEN 2014 has been approved by the Federation of the Royal Colleges of Physicians of the United Kingdom for 16 category 1 (external) CPD credit(s)

DATES for the DIARY
The current ISHEN president-elect Dr Radha K. Dhiman invites all delegates to the
17th ISHEN Symposium which will take place in India from 16th to 19th September 2016
SAVE THE DATES
ABSTRACTS

1 Miss Teresa García-Lezana
   Jordi Romero-Giménez, Lax Chavaria, Juan Geneser, Juan Cordoba
   Poster New animal model of episodic hepatic encephalopathy

2 Dr Goran Predy-Yamate
   Bernard Lanz, Corina Berset, Cristina Cadubal and Rolf Grueter
   Poster A pilot study bring physiology and metabolism into PET of the liver and brain of the BDL rat model

3 Dr Magdalena Zielinska
   Krzysztof Mlowski, Wojciech Hipier, Jan Albrecht
   Poster Diverses effects of histidine on the ADMA/NO pathway In the brain and blood of rats with Picrotoxin-induced hepatic failure

4 Miss Natalia Ovratiskova
   Philip Lang, Vitały Pozdeyev, Boris Gó and Dieter Häussinger
   Poster/Workshop Liver-specific glutamine synthetase knockout triggers hyperammonemia and oxidative stress in mouse brain

5 Miss Veronika Rayková
   Oliver Brassant, Corina Berset, Cristina Cadubal and Valéria A. McIn
   Poster Vivo longitudinal study of brain metabolism and edema in chronic hepatic encephalopathy in developing and adult rat brain

6 Dr Cristina Cadubal
   Veronika Rayková, Valéria A. McIn and Oliver Brassant
   Poster Brain glutamine, osmotides and osmosm in a model of chronic hepatic encephalopathy: In Vivo and longitudinal measurements using 1 H-MRS, DTI and immunofluorescence

7 Dr Marc Orià
   Andrea F. Garcia-Martinez R, Monforte RC, DE Chiera F, Shafii Y, Jalan R
   Poster P-AI-1 friend for some and enemies for others

8 Dr Christopher Rose
   Jimmy Huynh, Cristina R. Bosoi, Christian Parent-Robitaille, Mélanie Tremblay.
   Poster Buretanópsis normalizes brain edema in rats with bile duct ligation: Evidence for increased BBB permeability via MCKC1

9 Mr Varun Khetan
   Abeba Habtesion, Varun Khetan, Rajiv Jalan, Nathan Davies
   Poster Addition of benzoate to ornithine phenylacetate exploits additional ammonia-lowering pathways in a hyperammonemic model of chronic liver failure

10 Mrs Pamela Lockheed
    Abeba Habtesion, Varun Khetan, Rajiv Jalan, Nathan Davies
    Poster Albumin therapy ameliorates cerebral oedema in a model of chronic liver injury.

11 Miss Veronika Rayková
   Bernad Lanz, Valéria A. McIn, Oliver Brassant and Cristina Cadubal
   Poster STP Mrs in a rat model of chronic hepatic encephalopathy: in vivo Measurements of Brain Energy metabolism

12 Dr Karen Louise Thomsen
   H Grenaek, E Givand, L Hebbard, N Jessen, A Clouston, J George and H Vitstrup
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ABSTRACTS

1 NEW ANIMAL MODEL OF EPISODIC HEPATIC ENCEPHALOPATHY

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Introduction
Hepatic encephalopathy (HE) is a neuropsychiatric syndrome secondary to cirrhosis, caused by the entrance in the brain of toxic substances, which have been unable to be metabolized by the liver. Ammonia is the main factor in the pathogenesis of HE but there are other precipitant factors, such as inflammatory mediators, that worsen neurological manifestations. The use of appropriate animal models is a crucial step in the study of pathophysiological mechanisms behind the disorder and the development of new therapies. Although some animal models are available for the study of liver diseases, currently there is not a suitable one for episodic HE. The aim of our study is the development a new animal model able to reproduce the neurological impairments found in patients with episodic HE.

Materials & Methods
Portocaval anastomosis (PCA) was performed in rats in parallel with their sham-operated controls. Four weeks after surgery and in order to simulate episodes of HE, PCA rats were administered every two weeks during 5 months with: a single dose of LPS (3mg/Kg) to trigger inflammation, or ammonium acetate (55mM/Kg-min) to increase NH, in blood, a combined dose of LPS and ammonium acetate or vehicle (saline). Sham-operated rats were treated with vehicle following the same schedule. Twelve different reflexes (flexion, righting (A, B), grasping, placing reaction (A,B,C,D), equilibrium (A), corneal , auditory startle and head shaking) were checked in each animal at two time points, before drug infusion and 3 hours post infusion, and the number of positive reflexes was recorded.

Results
A significant loss of reflexes was observed 3h after drug infusion compared with baseline stage in the group of animals treated with ammonium acetate (post-infusion = 12; post-infusion = 9; P=0.0025) and the 36% of those rats lose at least 50% of reflexes after ammonia infusion. In contrast, the groups that were administered with LPS, a combined dose of LPS and ammonium acetate or vehicle maintained from 10 to 12 active reflexes after infusion (before treatment, all groups showed a minimum of 11 active reflexes).

Conclusions
This new model of HE reproduces disturbances in cognitive function after simulated episodes of HE with ammonia as a precipitant factor. This approach may be useful for the investigation of the pathophysiological mechanisms involved in episodic HE.

2 A pilot study bringing physiology and metabolism into PET of the liver and brain of the BDL rat model

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The aim of the present study, in progress, was to characterise liver, heart and regional brain energy metabolism non-invasively using 18FDG positron emission tomography (PET) in a rat model of hepatic encephalopathy, subsequent to 1H-MRS brain measurements at 9.4T in the same animals. Hepatic tracer kinetics were quantified by dynamic imaging of the liver following 18FDG (~50MBq) administration i.v. in sham-operated rats and bile duct-ligated (BDL) and hyperammonemia-positive rats, 8 weeks post-operation. In parallel, time-activity curves (TAC) of analog tracer concentrations in the inferior vena cava and heart were acquired until 40 min post-injection of 18FDG, at which time the brain was imaged during 15 minutes.

Preliminary results showed that the TAC of tracer in the vena cava – similar to that from the hepatic artery – was nearly identical between animal groups, indicating comparable availability of tracer to tissue regions of interest. Hepatic 18FDG kinetics (Figure, left panel) during the first 20 minutes were altered in BDL rats (symbol in brown) expressing prominent bile duct dilation (post-mortem) and increased liver volume (left panel image, acquired 10 min after 18FDG delivery). Bile duct ligation affected the glycolytic capacity in distant organs: (a) the left ventricle of the heart (middle panel) where 18FDG phosphorylation was significantly decreased, suggesting compromised motor and cognitive function that is a hallmark of hepatic encephalopathy. Experiments are currently underway to enable distinguishing hepatic blood perfusion from hepatic substrate utilisation.

3 Diverse effects of histidine on the ADMA/NO pathway in the brain and blood of rats with thioacetamide-induced hepatic failure

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Hepatic encephalopathy (HE) is invariably related to oxidative/nitrosative stress (ONS), and to changes in the NMDA receptor/nitric oxide (NO) pathway activity. An increase of brain asymmetric (NG, NG) dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthases (NOSs) in brain, which is subsequent to decreased dimethylarginine dimethylaminohydrolase (DDAH) activity, is one of recently discussed changes in the context of HE-evoked impairment of NO synthesis (Balasubramaniyan et al. 2011). Histidine (His) has been reported to alleviate HE-induced brain edema (Rao et al. 2010), by a mechanism encompassing attenuation of mitochondrial dysfunction and oxidative stress targeting astrocytes. We speculated that attenuation of oxidative stress by His may result in correcting the impaired DDAH/ADMA system imbalance and NO synthesis. In the present study TAA-induced acute liver failure (ALF) increased ADMA content by a mechanism related to the decrease of DDAH activity. An i.p. administration of His (100 mg/kg b.w.) reversed the decrease of brain ADMA which correlated with the increase of DDAH back to control level, and an increase of the total NOS activity. Activation of DDAH by His was confirmed by an ex vivo study in which His was applied directly to the homogenates derived from control and TAA rats. ALF in this model was also accompanied by increases of cyclooxygenase activity and TNF-α, the markers of the inflammatory response. However, these pro-inflammatory changes were not attenuated by His. TAA-induced ALF was associated with an increase of serum ADMA and a decrease of DDAH in the brain. However His did not correct these changes. The hypothesis currently explored is that His exerts its protective effect in brain either by antioxidant activity or indirectly, subsequent to its conversion to histamine. Supported by the National Science Centre grant No 2013/09/B/NZ4/00536 The authors declare no conflict of interests.

4 Liver-specific glutamine synthetase knockout triggers hyperammonemia and oxidative stress in mouse brain

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Introduction: Ammonia is a major toxin involved in pathogenesis of hepatic encephalopathy (HE). In addition to ammonia detoxification by the urea cycle, hepatic glutamine synthetase plays an important role for maintenance of ammonia homeostasis. In the liver, expression of glutamine synthetase (GS) is restricted to so-called “scavenger cells” which comprise a small population of hepatocytes. The scavenger cells are localised to the hepatic venous outflow and were postulated to eliminate residual blood ammonia escaping the portal urea-synthesizing compartment (Häussinger Eur J Biochem 1983; 133:269-275; Biochem J 1990; 267:281-290). In order to analyse the role of hepatic glutamine synthetase for systemic ammonia detoxification and ammonia-induced cerebral oxidative stress, we generated a liver-specific glutamine synthetase knockout mouse.

Methods: A liver specific GS-knockout mouse was generated by using the Cre-loxP system in which the glutamine synthetase gene is flanked by loxP sites and Cre recombinase expression is driven under albumin promoter control. Results: In transgenic AlbCre/GS-LoxP mice, GS protein was not detectable in liver but its expression remained unchanged in brain as shown by Western-blot and immunofluorescence analysis. Blood ammonia levels were significantly elevated in GS KO-mice. Tissue architecture was preserved in GS knockout (KO) mice and blood enzyme indicators indicative for liver damage (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) were not significantly affected. Hyperammonemia in GS KO-mice was associated with increase of oxidative stress markers such as RNA oxidation and protein nitrilation in cerebellum, hippocampus and somatosensory cortex. AlbCre/ GS-LoxP animals showed altered locomotor behaviour and had a significantly reduced life span compared to GS-WT mice.
ABSTRACTS

Conclusions: The results demonstrate that GS in perivenous hepatic scavenger cells is critical for maintenance of systemic ammonia homeostasis. The study shows that chronic hyperammonemia in the absence of liver damage is sufficient to induce cerebral oxidative stress associated with altered behavior and establishes liver-specific GS KO-mice as a unique model to studies on the pathophysiology of chronic hyperammonemia and hepatic encephalopathy.

5 IN VIVO LONGITUDINAL STUDY OF BRAIN METABOLISM AND EDEMA IN CHRONIC HEPATIC ENCEPHALOPATHY IN DEVELOPING AND ADULT RAT BRAIN

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Introduction
Adults with chronic liver disease (CLD) display neurocognitive deficits known as Spectrum of Neurocognitive Impairment in Cirrhosis (SONIC). There is increasing evidence that children with CLD may also present neurocognitive deficits early in life [1]. Adults recover from hyperammonemia (HA) when NH₄⁺ blood levels return to normal values, while children exposed to HA early in life show life-long deficits [2], suggesting that there is a developmental window of susceptibility. To assess the age-dependent vulnerability of the brain to CLD-induced insults, we compared the neurometabolism, histology, and serum biochemistries of adult and pups bile duct ligated (BDL) rats.

Methods
Adult and 21 day (corresponding to childhood [3]) Wistar rats were BDL (CLD model [4]) and scanned before and at post-operative weeks 2, 4, 6, 8. ¹H magnetic resonance spectroscopy (MRS) and diffusion tensor imaging (DTI) were performed on a 9.4T system. Voxel of 11.2mm³ for ¹H MRS in the hippocampus was measured by SPECIAL sequence (T₂=2.8ms) [5], metabolite concentrations were calculated by LCModel. DTI was performed with EPI sequence [6] and diffusivity values were derived from the tensor. ADC (apparent diffusion coefficient) was measured in cortex, striatum and hippocampus. Immunohistochemistry on brain tissue was performed using astrocytic and water channel markers (GFAP, AQP4). Serum was collected to measure NH₄⁺ and other biochemical parameters.

Results and Discussion
Pups displayed more significant neurometabolic and biochemical changes than adult BDL rats for all parameters (Fig1). A more pronounced increase of brain glutamine which was associated with marked edema in spite of ongoing osmoregulation as measured by decreased myoIn. Enhanced expression of AQP4 in cerebral microcapillaries was more noticeable in pups than adults. We observed a greater decrease in brain neurotransmitters and antioxidants in pups coupled with a marked increase in lactate, consistent with altered energy metabolism.

Conclusion
We conclude that osmotic and metabolic changes are greater in pups than adults, and are associated with altered AQP4 expression, pointing to increased BBB permeability. How these two processes are linked and contribute to edema and neurocognitive changes remains to be determined.

References

6 BRAIN GLUTAMINE, OSMOLYTES AND EDEMA IN A MODEL OF CHRONIC HEPATIC ENCEPHALOPATHY: IN VIVO AND LONGITUDINAL MEASUREMENTS USING ¹H MRS, DTI AND IMMUNOFLOUORESCENCE

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Chronic liver disease (CLD) is characterized by an array of cognitive and fine motor deficits labeled as hepatic encephalopathy (HE). Our aim was to assess, in vivo and longitudinally on a 9.4T MRI system, several pathogenic mechanisms involved in HE (osmoregulation, neurotransmitters, oxidative stress, energy dysfunction, cell swelling) using ¹H-MRS, Diffusion Tensor Imaging and immunofluorescence.

Wistar rats were bile duct-ligated (BDL) and scanned before and each 2 weeks post-BDL (8 weeks). The SPECIAL spectroscopy sequence (TE=2.8ms) was used (hippocampus), while the ADC-apparent diffusion coefficient was measured in cortex, striatum and hippocampus. Brain tissue morphology was assessed by immunofluorescence using astrocytic (GFAP) and water channel (AQP4) markers.

Glutamine increased linearly over time (200%) while plasma ammonia increased to 109±37µM at 8 weeks (Fig1). As a compensatory effect, other brain osmolytes decreased: myoIn (-30%), followed by taurine and choline (-10% to -30%) as well as ascorbate (-13%), a metabolite involved in energy metabolism but recently described in osmoregulation and neuromodulation. Among neurotransmitters, glutamate, aspartate and GABA were progressively decreased (-10% to -30%). We also noticed a decrease of the antioxidants ascorbate and glutathion (-9%). ADC values showed a slight increase over the first 8 weeks post-BDL.

Brain tissue morphology was significantly affected 8 weeks post BDL, with great variations between individuals. The most severely affected animals showed a strong vacuolization of whole brain tissue, which was particularly intense in cortex, hippocampus and striatum. While AQP4 was increased in microcapillaries of the less affected animals, AQP4 was decreased in animals most severely affected but demonstrated that their CNS vacuolization specifically affected brain parenchyma but not microcapillaries. GFAP immunostaining showed astrocyte swelling in cortex and hippocampus of the less severely affected animals, suggesting that mild edema develops in spite of ongoing osmotic regulation. In contrast, GFAP was clearly detectable around the vacuoles observed in the brain of the most severely affected animals.

Our work suggests that prior to appearance of severe neurological signs in CLD, the osmotic imbalance created by continuous increase of glutamine may be compensated by a concomitant decrease of other idiosyncratic osmolytes resulting in minimal brain edema and glutamine may be the main cause.
ABSTRACTS

Aims: Using an animal model for chronic liver failure (CLF) and ACLF we aimed to determine the expression of PAI-1 in different tissues.

Methods: Cortex, cerebellum, liver and kidney samples were collected from 6 weeks bile duct ligated rats (BDL) (CLF), BDL+LPS (0.3 mg/Kg, 3h iv infusion) (ACLF) and sham-operated rats (control) (n=6/group). RNA was isolated and pellets were partially re-extracted, precipitated, DNAse-digested and cleaned. 1ug of RNA/sample was retro-transcribed and run on RT2-PCR-array rat cellular senescence plates. Ammonia and TNFα were assessed in plasma. PAI-1 Immunohistochemistry was assessed in tissue.

Results: PAI-1 was over expressed in each model and in each tissue compared to controls. The Fold Change of PAI-1 compared with Sham is summarized in Table 1.

Table 1.

<table>
<thead>
<tr>
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<th>PAI-1</th>
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<tbody>
<tr>
<td>Liver</td>
<td>BDL</td>
</tr>
<tr>
<td>Kidney</td>
<td>7.2±4.5</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>-</td>
</tr>
<tr>
<td>Cortex</td>
<td>2.03</td>
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</table>

Conclusions: Dysregulation of PAI-1 within organs was observed in CLF trying to repair the injured liver. After exacerbation of inflammatory response, oxidative stress and TNFα led to an overexpression of PAI-1 inducing kidney dysfunction and the activation of senescence in the brain. Concrete knowledge of PAI-1 expression will be helpful to design tissue-specific therapeutic approaches in ALCF.

8

BUMETANIDE NORMALIZES BRAIN EDEMA IN RATS WITH BILE DUCT LIGATION: EVIDENCE FOR INCREASED BBB PERMEABILITY VIA NKCC1

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Background: Brain edema is a serious complication associated with hepatic encephalopathy (HE) due to chronic liver disease. An increase in BBB ion permeability (increase uptake of ions and accompanied by water fluxes towards the brain) can occur across an intact BBB through alterations in transport mechanisms. Endothelial cells that comprise the BBB secrete up to 40% of brain interstitial fluid as they transport Na+ and K+ has very similar ionic properties to K+ (similar ionic radius and diffusion coefficient) and can be transported through K+ channels and cotransporters, implying that hyperammonemia could result in BBB hyperpermeability. An increase in BBB permeability via transport via NKCC1 (NKCC1) has shown to promote brain edema and astrocyte swelling under pathophysiological conditions such as ischemia. Aim: To study the BBB integrity (vasogenic vs cytotoxic) and the role of NKCC1 in the pathogenesis of brain edema in cirrhotic rats. Methods: Two distinct animal models of chronic liver failure and HE are used in the present study: 1) biliary cirrhosis model (6 weeks bile duct ligation (BDL)), 2) portacaval shunt model (4 weeks portacaval anastomosis (PCA)). Both models develop hyperammonemia however brain edema is only observed in BDL. BBB breakdown was assessed by measuring brain extravasation of Evans blue and sodium fluorescein (injected i.v). Expression of BBB tight junction proteins (occludin, claudin-5, ZO-1 and ZO-2) were assessed by Western blot. Bumetanide was administered (i.p) for 10 days in BDL and BDL SHAM. Brain water content was measured in the frontal cortex using the specific gravimetric method. Levels of brain NKCC1 mRNA were evaluated by RT-PCR in cerebral microvessels. Results: Extravasation of Evans blue and sodium fluorescein was not detected and there was no significant change in all tight junction protein levels measured in both BDL and PCA models. Brain water content was reduced in bumetanide-treated BDL rats compared to control (77.66±0.15% vs 78.12±0.21%). In brain microvessels, NKCC1 mRNA increased in BDL rats compared to BDL SHAM (0.78±0.09 vs 1.92±0.42) whereas no change was found in PCA compared to PCA SHAM (1.72±0.52 vs 1.53±0.23). Conclusions: BDL rats did not demonstrate a change in BBB integrity or in expression of BBB tight junction proteins. This suggests brain edema in BDL is not of vasogenic origin. Furthermore, since brain edema was only observed in BDL rats (vs PCA), this implies additional factors aside ammonia, are involved in the pathogenesis of brain edema. Moreover, an increase of NKCC1 mRNA and an attenuation of brain edema following bumetanide treatment were demonstrated in BDL rats suggesting NKCC1 plays a role in the development of brain edema in chronic liver disease. Furthermore, these results demonstrate the potential therapeutic use of bumetanide for the treatment of HE.
ABSTRACTS

9 Addition of benzoate to ornithine phenylacetate exploits additional ammonia-lowering pathways in a hyperammonaemic model of chronic liver failure.

Abstract
Ornithine Phenylacetate (OP) has been shown to be effective in lowering ammonia in bile duct ligated (BDL) rats, hepatic devascularized pigs, and in patients with chronic liver failure. The proposed mechanism is that ornithine promotes glutamine production (incorporating ammonia), which then conjugates with phenylacetate (PA) to form phenylacetylglutamine (PAGly) which is then excreted (fig. 1). Further investigation has also found that a second conjugated product of phenylacetate, phenylacetylglycine (PAGly), was also excreted in comparable quantities to PAG. PAGly production limits the efficacy of ammonia removal by PAGN, in which two amino nitrogens are excreted for each conjugation with PA. Given this observation, we conducted a study to test whether it is possible to restrict this newly discovered PAGly pathway, thereby increasing the efficacy of PAGN mediated ammonia removal. For this benzoate was utilised to promote hippuric acid production from glycine to provide an alternate excretion pathway, but still reducing the overall metabolic nitrogen load. The hypothesis tested was that the addition of benzoate to OP therapy may result in greater attenuation of hyperammonaemia in models of liver injury.

Methods:
Male Sprague-Dawley rats (250g) underwent either BDL (or sham operated control – SHAM) surgery. At day 23, animals were given OP, ornithine plus benzoate (OB, 1:1), or ornithine phenylacetate plus benzoate (OPB, 1:1:1 ratio) at 2g/kg/day IP for 5 days. Samples were collected under terminal anaesthesia on day 28. Therapy test groups were given a high protein, pro-hyperammonaemic diet from day 21 (NH3 groups). The 6 animal groups were: Sham, BDL, +saline placebo), BDL+NH3, BDL+NH3+OP, BDL+NH3+OB, and BDL+NH3+OPB.

Plasma biochemistry (ALT, AST, bilirubin, ammonia, albumin, creatinine, urea) were measured using a Cobas Integra system, urinary hippuric acid was determined via the pyridine colourimetric method.

Results:
The use of benzoate as a therapy in this chronic BDL model was safe and well tolerated by the animals. There was no significant effect on the standard biochemical measures (AST, ALT, Bilirubin, Urea, Albumin, creatinine), indicating that there was no change in the basal liver function in any treatment group compared with placebo. OB therapy alone did not lead to a reduction in ammonia, however the addition of benzoate to OP did result in a further lowering in plasma levels (OPB 114µM vs OP 130.9µM) though this difference was not significant. As expected, urinary hippuric acid levels rose in both groups treated with benzoate.

Conclusion:
These results show that OP when given in conjuction with benzoate, further attenuates hyperammonaemia in the BDL model of chronic liver injury. Although further studies are required to determine an optimal dosing ratio to maximise this effect, this may prove to be a significant improvement in the efficacy of this treatment.

10 Albumin therapy ameliorates cerebral oedema in a model of chronic liver injury.

Background
Albumin has previously been demonstrated to be effective in reducing cerebral injury in stroke victims, most probably due to its anti-oxidant properties. It is often administered to liver disease patients with hypotension/hypovolemia, with some studies showing additional benefits in terms of reduced mortality and morbidity. Considering these reported effects, in this study we have examined whether addition of albumin to a rodent model of chronic liver injury ( bile duct ligated rat model, BDL), would have a positive benefit on cerebral oedema when administered at a late stage in the development of liver injury.

Methods
Male Sprague-Dawley rats (250g) underwent either BDL (or sham operated control – SHAM; n=7 per group) surgery. On days 26 and 27, BDL animals were given commercially available albumin (20% w/v, 5g/Kg/day IP), or saline placebo. On day 28 the animals were fasted and had catheters placed into the left carotid artery and portal vein for sampling and pressure measurements. Blood and tissues were then collected at the termination of the experiment.

Results
As was expected, treatment of the BDL animals with albumin resulted in a significant increase in plasma albumin (p<0.001 BDL+Alb vs BDL) and total protein levels above those observed in the sham cohort (BDL 52.84, Sham 52.18, BDL+Alb 62.34 g/L, p<0.05). Administration of albumin significantly improved mean arterial pressure to near normal levels compared with the BDL placebo group (BDL 78.9, BDL+Alb 95.0, sham 104.1; p<0.05 BDL vs BDL+Alb). There was also a trend towards reduction in portal pressure, although this was not significant. Brain water content was significantly lower in the albumin treated BDL animals (BDL+Alb 79.6, BDL 80.4; p<0.01). Other markers of liver injury (ALT, AST etc) remained unchanged between the BDL placebo and albumin treated groups.

Discussion
Though it is well established that administration of albumin confers vascular stability in subjects with chronic liver disease, in this study we have also demonstrated the additional amelioration of brain water (which is a surrogate marker for cerebral oedema), even when administered following the development of liver injury. It is well known that albumin has anti-oxidant and anti-inflammatory properties (including the ability to bind endotoxins and other mediators), and it is possible that this effect is mediated in this way considering the known effects of inflammation in the occurrence of brain swelling. This study provides additional evidence that albumin has additional therapeutic properties beyond that of a simple volume expander.

11 31P MRS IN A RAT MODEL OF CHRONIC HEPATIC ENCEPHALOPATHY: IN VIVO MEASUREMENTS OF BRAIN ENERGY METABOLISM

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3Service of Biomedicine, University Hospital of Lausanne, Lausanne, Vaud, Switzerland.
4Centre d’Imagerie Biomedicale (CIBM), Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Vaud, Switzerland.

To date the mechanism leading to brain edema is still unclear in chronic liver disease (CLD). Whether brain energy metabolism is affected is of some debate. We have previously shown in rats with biliary cirrhosis that increased CNS NH3+ generates a rise in the osmolyte glutamine (Gln) followed by an osmotic compensation as shown by the gradual decrease of other brain osmolytes. In spite of this apparent osmoregulation, low grade brain edema is present as demonstrated by astrocyte swelling and A þ-D accurate diffusion coefficients. The aim was to measure in vivo and longitudinally brain energy metabolism in bile duct ligated (BDL) rats using 31P MRS (magnetic resonance spectroscopy) together with 1H MRS and DTI (diffusion tensor imaging) for the measurement of brain oedema and metabolism.

Wistar adult rats were BDL, scanned before BDL and weekly thereafter for 8 weeks. In vivo localized 1H and 31P MRS was performed on a 9.4T system. Metabolite concentrations were calculated using water as internal reference for the 1H data and phosphocreatine (PCr) for the 31P data. DTI was performed and diffusivity values (ADC coefficient) were measured in cortex, striatum and hippocampus.

BDL rats showed increased plasma NH3+ of 109±37 µM (versus 52±8µM in sham) (Fig1). At 8 weeks after BDL we measured a 200% increase of brain Gln (-30%), followed by taurine and choline (-10% and -30%) as well as creatine (-13%), a metabolite involved in energy metabolism but recently described in osmoregulation and neuromodulation. PCr, a metabolite involved in energy metabolism, was constant over time while lactate showed a small increase of 10%. ADC values showed a mean increase (+10%) over the first 8 weeks post-BDL, suggesting that mild edema develops in spite of ongoing osmoregulation. 31P MRS data showed a gradual decrease of yATP/PCr ratio, meaning that there was a gradual decrease of yATP (-10%) since PCr values were constant over time.
Our work suggests that the osmotic imbalance created by the continuous increase of Gln may be partially compensated by a concomitant decrease of other idogenic osmolytes resulting in minimal brain edema. We studied ureagenesis, an essential hepatic metabolic function with importance for whole-body nitrogen homeostasis, in a rodent model of diet induced NASH. We examined the urea cycle enzyme mRNAs in liver tissue, the hepatocyte urea cycle enzyme proteins and the in vivo Capacity of Urea-Nitrogen Synthesis (CUNS).

**Methods:** Early NASH decreased the urea cycle mRNAs to an average of 60% and the ornithine transcarbamylase protein to 10% while the CUNS remained unchanged. Advanced NASH further decreased the carbamoyl phosphate synthetase protein to 63%, and in addition decreased the CUNS by 20% (from 5.65 ± 0.23 to 4.58 ± 0.30 mmol x (min x 100 g) -1; P = 0.01).

**Conclusion:** Early NASH compromised the genes and enzyme proteins involved in ureagenesis, while advanced NASH resulted in a functional reduction in the capacity for ureagenesis. The pattern of urea cycle perturbations suggests a prevailing mitochondrial impairment by NASH. The decrease in CUNS has consequences for the ability of the body to adjust to changes in the requirements for nitrogen homeostasis e.g. at stressfull events. NASH, thus, in terms of metabolic consequences is not an innocuous lesion.

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glutamine (Gln) as a product of ammonia detoxification is further metabolized by mitochondrial glutaminase (GLS), which is thought to play a central role in the generation of excitotoxic glutamate (Glu). A recent study reported that the ratio of glutamate to glutamine was increased in the liver, brain, and microglial cells, and decreased in the serum of cirrhosis patients with HE. HE+ rats had a significantly lower ratio than HE− rats. The authors concluded that glutamine may be a potential therapeutic target to prevent and reverse sarcopenia in cirrhosis.

Supported by the National Science Centre (NCC) grant 2013/09/B/NZ4/00536. The authors have declared that no conflict interests exist.

16 INCREASED MYOSTATIN TRANSCRIPTION BY HYPERAMMONEMIA IN CIRRHOSIS IS MEDIATED BY REDUCED \( \beta \)-Catenin ACTIVATION.

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Background. Hyperammonemia induction of NFkB mediated transcriptional upregulation of myostatin, a TGFβ superfamily member. Myostatin decreases skeletal muscle mass protein synthesis and conversely, skeletal muscle ribosomal biogenesis and protein synthesis are stimulated by \( \beta \)-catenin/T cell transcription factor (TCF) signaling. Therefore we hypothesized that hyperammonemia affects functions by inhibiting \( \beta \)-catenin signaling and increased myostatin expression.

Methods. Differentiated C2C12 murine myotubes were exposed to 10 mM ammonium acetate to induce hyperammonemia using protocols standardized in our laboratory. \( \beta \)-catenin protein levels and signaling was activated by inhibition Gsk3β using 1 µM bromoindirubine-3-oxime (BIO). Myostatin mRNA and protein expression. Conversely, \( \beta \)-catenin activation with BIO inhibited the hyperammonemia-induced upregulation of myostatin. Chronic exposure to hyperammonemia reduced the levels and the transcriptional activity of \( \beta \)-catenin.

Results. Myostatin transcriptionally upregulated myostatin mRNA and protein expression. Conversely, \( \beta \)-catenin activation with BIO inhibited the hyperammonemia-induced upregulation of myostatin. Chronic exposure to hyperammonemia reduced the levels and the transcriptional activity of \( \beta \)-catenin. These results provide evidence for the role of pro-inflammatory cytokines in the pathogenesis of cirrhosis and HE in ACLF patients. DTI derived metrics and H-MRS are very good non-invasive tools for understanding the pathogenesis of CE in ACLF.

Key Words: Acute-on-chronic liver failure, Diffusion tensor imaging, Proinflammatory cytokines, Hyperammonemia, H-MR spectroscopy.

18 Astrocitary NMDA receptors mediate downregulation of Kir4.1 potassium channels induced by glutamate and TNF-α in cultured astrocytes and in HE-affected rat brain in vivo

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Background. Though hyperammonemia is known to be a major factor in the pathogenesis of cerebral edema (CE) and hepatic encephalopathy (HE) in liver failure, recent reports suggest that pro-inflammatory cytokines also contribute significantly to the developing elevated pro-inflammatory cytokines with CE in acute-on-chronic liver failure (ACLF) are lacking.

Aims: This study looked at the relationship of pro-inflammatory cytokines with glutamate/glutamine ratio (Glx) on \( ^{1}H \)-MR spectroscopy (\( ^{1}H \)-MRS), a measure of cerebral ammonia elevation, and diffusion tensor imaging (DTI) derived metrics (MD and mean diffusivity) in patients with CE of ACLF patients.

Methods: Seventeen patients with ACLF and 14 controls were included. Serum pro-inflammatory cytokines (IL-6 and TNF-α), blood ammonia and Glu were measured in both groups along with MRI studies (\( ^{1}H \)-MRS and DTI). Correlations between cytokines and MR derived metrics were assessed using Pearson’s correlation coefficient.

Results: Levels of cytokines, blood ammonia and Glx were significantly increased in ACLF patients as compared to controls (p<0.001). Significant positive correlation was present between cytokines and Glx (r=0.667;p=0.003) for TNF-α and (r=0.522;p=0.04) for IL-6 as well as with spectroscopy voxel (SV) derived CS (r=0.578;p=0.015) for TNF-α and (r=0.681;p=0.003) for IL-6, while a negative correlation was noted with SV derived MD (r=0.506;p=0.038) for TNF-α and (r=0.619;p=0.008) for IL-6.

Conclusions: These results provide evidence for the role of pro-inflammatory cytokines in the pathogenesis of CE in ACLF patients. DTI derived metrics and H-MRS are very good non-invasive tools for understanding the pathogenesis of CE in ACLF.

Supported by the National Science Centre (NCC) grant 2013/B09/B/NZ4/00536. The authors have declared that no conflict interests exist.
Results: By transcriptome analysis we identified 945 genes upregulated and 602 genes downregulated in patients with liver cirrhosis and HE compared to controls. These gene expression changes were accompanied by a significant upregulation of 25 different miRNA species as revealed by Agilent\textsuperscript{a} array analysis. A strong tendency towards upregulation could be confirmed for 7 miRNA species using miQPCR. Bioinformatic analysis revealed that many genes downregulated in patients with cirrhosis and HE are potential targets of concurrently upregulated miRNAs. These genes were related to learning and memory processing, sleep/wake rhythm and microglia activation.

Discussion: The present findings point to a potential role of miRNA expression changes for gene expression changes in brain of cirrhotic patients with HE.

20 Ammonia-induced senescence in cultured rat astrocytes and in human cerebral cortex in hepatic encephalopathy

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Introduction: Recent studies indicate that impaired cognitive functions in patients with liver cirrhosis and hepatic encephalopathy (HE) may not completely resolve after an attack of acute HE (Bajaj et al., Gastroenterology 2010; 138: 2332-40; lin Gastroenterol Hepatol 2011; 9:181-183). Since synaptic transmission strongly depends on astrocytes and astrocyte dysfunction is a hallmark of HE, we tested for sustained astrocyte dysfunction indicated by senescence biomarkers in ammonia-treated cultured astrocytes and in post-mortem brain samples from patients with liver cirrhosis with and without HE.

Methods: Astrocyte senescence was assessed by measuring DNA synthesis as a surrogate marker for proliferation. Senescence biomarkers were examined by immunofluorescence (b-galactosidase activity, nuclear accumulation of phosphorylated p53 or p21) and real-time-PCR (GADD45a, p53, p21). Post-mortem brain samples of patients with liver cirrhosis with and without HE and controls free from neurological diseases were obtained from the Australian Brain Donor Programs NSW Tissue Resource Centre (University of Sidney).

Results: Treating astrocytes with NH\textsubscript{4}Cl inhibited proliferation in a time- and dose-dependent manner up to 50% (5mMol, 72h) and strongly increased senescence-associated b-galactosidase activity. NH\textsubscript{4}Cl (5mMol, 72-treatment) induced nuclear accumulation of the cell cycle-regulatory transcription factor p53 accompanied by increased transcription of cell cycle inhibitory genes GADD45a and p21. Increased nuclear accumulation of p53 and GADD45a and p21 were also found in post-mortem brain samples of patients with liver cirrhosis and HE but not without HE.

Discussion: The present study suggests a role for astrocyte senescence in the pathogenesis of hepatic encephalopathy.

21 Dimethylarginine Dimethylaminohydrolase-1 (DDAH-1), a Key Regulator of Brain eNOS Activity, is Modulated by microRNA 30 in vitro

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Background and Aims: Chronic hyperammonemia is associated with reduced brain NO and cGMP availability, associated with microvascular integrin alterations and decreased cerebral blood flow. DDAH-1 is a key regulator of eNOS activity, through metabolism of the endogenous inhibitor of eNOS – ADMA. We have previously shown that treatment of bile duct ligated (BDL) rats with ornithine phenylacetate leads to reduced brain ADMA and increased brain DDAH-1 expression indicating a deceleration of microvascular integrin alterations and increased cerebral blood flow.

Methods: Bioinformatics/Luciferase reporter assays: Highly conserved miRNA binding sites in the DDAH-1 3'UTR were identified using the Targetscan and microRNA databases. The human DDAH-1 3'UTR, and short (~50bp) sequences containing predicted miRNA binding sites, were cloned into the pMIRReport (Lifetech) vector. Luciferase output was determined 24 hours following transfection into HEK293T cells.

Transfection of miRNA mimics: miRNA mimics (Qiagen) for candidate miRs were transfected into HEK293T cells, along with scrambled control. DDAH-1 protein expression was determined 24 hours following transfection.

Results: Binding sites for miR-30 are present in DDAH-1 3’UTR and highly evolutionarily conserved. Reporter assay demonstrate that the DDAH-1 3’UTR significantly decreased luciferase expression compared to the pMIRReport control (p<0.01), as did a shorter 50bp sequence containing the miR-30 binding site (p=0.01), although this repression was lost by site-directed mutagenesis of the miR-30 site. Transfection of miR-30 mimic led to a significant decrease in DDAH-1 protein expression in HEK293T cells compared to scrambled control (p<0.01).

Conclusions: DDAH-1 undergoes post-transcriptional regulation by miR-30. Targeting brain miR-30 in HE, with anti-miR vectors, may lead to increased DDAH-1 availability, decreased ADMA and improved brain eNOS activity in HE.

22 Muscle glutamine synthetase and infection

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Patients at the end stage of liver disease frequently have bacterial infections and become the first cause of death of these patients. Moreover, in cirrhosis the urea synthesis capacity is impaired as well as capacity of glutamine synthetase to produce glutamine leading to increased ammonia levels with the progression of the disease. Glutamine is a multifunctional amino-acid that is important in regulating ammonia metabolism, protein synthesis, immune function and gut integrity. We decided to determine the effect of knocking-down muscle GS (GS-KO/M) in the mice in gut integrity assayed by assessed basal plasma levels and in impaired urea synthesis with a sub-lethal dosage of paracetamol.

Methods: GS-KO/M mice were obtained by a selective elimination of GS expression in striated muscle. 4 groups of animals were studied: FVB, FVB normal (WT), FVB with GS-KO/M received paracetamol (IP 250 mg/kg) to induce liver failure (ALF), or saline (IP) (n=12 in each group). Plasma was measured for: ammonia and standard biochemical markers (AST, ALT, bilirubin, urea, lactate, glucose, creatinine; COBAS-Roches). Brain water and ammonia were measured. TNF-α was determined by ELISA in liver tissue homogenates. Endotoxin plasma was assessed by LAL test (Endosafe,Charles River).

Results: Endotoxin plasma levels were increased in GS-KO/M mice compared to WT (1727±296 vs. 852±135 eM/mL; n=3) and further increased after APAP (WT 1197±110 vs. 1604±240 eM/mL; p<0.01) and in GS-KO/M (299.5 ± 7.5 µmol/L) (p<0.05 vs. GS-KO). Severity of liver injury measured using ALT, AST and lactate were similar between groups but hepatic TNF-α was higher in the GS-KO/ALF versus WT (6.7 ± 1.6 vs. 4.0 ± 0.5 ng/mg protein; p< 0.05). Brain water was increased in GS-KO ALF vs. WT ALF (79.8 ± 0.5 vs. 78.7 ± 0.4%; p< 0.05).

Conclusions: The preliminary results of this study provides direct evidence for the importance of muscle GS in regulating gut integrity and ammonia levels and hepatic inflammation in patients with liver cirrhosis. GS is important in regulating gut integrity and ammonia levels as well as regulating the progression of the disease. Glutamine supplementation cannot be undertaken due to its ammoniagenic effect.

Acknowledgments: Professor Janeway, and Drs. I. S. Sherlock, and E. E. Callahan.

23 Activation of senescence pathways in chronic and acute-on-chronic liver failure rat brain

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Background: Cirrhotic patients can suffer from a neurodegenerative process related to hepatic encephalopathy (HE). Reduction in brain size is associated with the number of overt HE episodes and may persist after liver transplantation. We hypothesize that senescence that is consequence on episodes of HE may be due to hyperammonemia and/or activation of inflammation, which often results in acute-on-chronic liver failure (ACLF).

Aims: Using an animal model for chronic liver failure (CLF) and ACLF we aimed to characterize molecular signatures and assess the relationship between nitrogen metabolism, inflammation and neurodegeneration.

Methods: Cortex and cerebellum samples were collected from 6 weeks bile duct ligated rats (BDL) (CLF), BDL+LPS (0.3 mg/Kg, 3h i.v infusion) (ACLF) and sham-operated rats (control) (n=6/group). RNA was isolated and pellets were partially re-extracted, precipitated, DNAse-digested and cleaned. 1ug of RNA/sample was retro-transcribed and run on RT-qPCR-array rat cellular senescence plate. Ammonia and TNFα were assessed in plasma.
Results: Senescence-associated genes were differentially expressed in each model and in each brain region compared to controls. The genes are summarized in Table 1.

BDL rats showed higher ammonia and TNFα levels compared with control rats 71.5±22.4 and 7.2±4.5 pg/mL. BDL+LPS ammonia remained stable but TNFα increased to 789.5±212.9 pg/mL.

Conclusions: Dysregulation of senescence pathways within brain regions was observed CLF. In BDL, oxidative stress (NOX4) and cyclin D (CDKN2B) pathways were affected in the cortex and the cerebellum respectively. When CLF was exacerbated by LPS, the dysregulation of further senescence pathways was observed suggesting that precipitating factors and consequent inflammation might induce neurodegeneration in the ALCF.

24 Ammonia acts as a damage associated molecular pattern (DAMP) producing multi organ injury and inflammation through a Toll-Like-Receptor-4 (TLR4) dependent pathway

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Liver Failure Group, Institute for liver and Digestive Health

Background:
Ammonia is thought to be central in the pathogenesis of hepatic encephalopathy (HE) but recent studies suggest that ammonia may have pleiotropic effects and be involved in the pathogenesis of portal hypertension, immune dysfunction and cachexia of liver failure. The mechanisms of these deleterious effects of ammonia remain uncertain. In previous studies, a close synergy between ammonia and inflammation has been demonstrated but the mechanism of this synergy is uncertain. TLR4 is a ubiquitous receptor for several ligands and signalling through this is associated with activation of NFkB and cytokine production. The aims of this study were to test the hypothesis that ammonia acts as a DAMP, which may induce multi-organ dysfunction acting through the TLR4 mediated pathway. We also aimed to determine whether TLR4 in the brain was important in modulating brain ammonia metabolism and swelling.

Materials and Methods: 4 groups of mice were studied: Wild-type control (WT, n=10), WT-hyperammonemia (WT-NH3, n=10), TLR4-/-control (TLR4-KOC, n=6), TLR4-/- hyperammonemia (TLR4-/-NH3, n=15). Hyperammonemia was induced with addition of 0.2M ammonium chloride to drinking water, 3 days after which they were sacrificed and plasma and brain collected for biochemical and histological analysis.1HNMR of the isolated cortex was performed to study the brain metabolism of ammonia.

Results:
Induction of hyperammonemia to pathophysiological concentrations (340.9 ± 32 WT-NH3, 247.6 ± 18.7 TLR4-/-NH3 ) resulted in significant liver (albumin 30.3±0.9 WT-NH3 vs 26.7±0.9 TLR4-/-NH3 , urea 8.3±0.1 WT-NH3 vs 7.2±0.4 TLR4-/-NH3 ) and renal (creatinine) injury (11.8±0.7 WT-NH3 vs 7.19±0.3 TLR4-/-NH3 ) in WT mice, which was significantly abrogated in the TLR4-KO mice (p<0.006, p<0.001, p<0.0001 respectively). Hyperammonemia resulted in a significant increase in brain water in WT animals, which was significantly reduced in the TLR4-KO cohort (p=0.05). 1H NMR spectroscopy of the cortical brain revealed that lactate and glutamine were significantly lower in hyperammonemic WT vs groups TLR4-/- (p<0.05, p<0.004 respectively).

Conclusion: The results of this study strongly support the concept that ammonia acts as a DAMP producing multiple organ injury and inflammation through a TLR4 dependant pathway. For the first time, the data from this study show that TLR4 modulates brain ammonia metabolism and accumulation of lactate and glutamine leading to brain swelling explaining the synergy between ammonia and inflammation in the pathogenesis of HE. We conclude that TLR4 inhibition may reduce ammonia induced brain swelling and is therefore an important target of therapy.

25 Comorbid disease negatively affects psychometric tests for hepatic encephalopathy

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Background: Psychometric testing is used for diagnosing covert hepatic encephalopathy (CHE); a debilitating condition that slows cerebral activity in approximately half of patients with liver cirrhosis. Other chronic diseases, such as heart failure (HF), chronic kidney disease (CKD) and diabetes (DM) are also associated with impaired cognition. The effect of co-morbid disease on psychometric tests used to diagnose CHE is unknown. Aim: The aim of our study was to examine effects of chronic diseases on two different psychometric tests used for diagnosing CHE. The Portosystemic Encephalopathy syndrome test (PSE) test and the Continuous Reaction Time (CRT) method. Patients and methods: The two tests were applied in 15 patients with CKD prior to dialysis, 15 patients with deregulated DM and 13 patients with HF in an outpatient setting. The results were compared with data from 18 healthy age matched persons and 17 patients with liver cirrhosis. The PSE test is a paper pencil test that primarily evaluates psychomotor speed whereas the CRT method is a 10 minutes computerized test that describes arousal functions by measuring reaction time 150 times within 10 minutes. Reaction time instability (CRTIndex < 1.9) in a liver patient indicates CHE. Contingency table analysis and Fisher’s exact test was used to compare results. Results: Ten in 17 cirrhosis patients (60%), and none in the control group, had abnormal PSE results (p<0.0001). Tree (20%) in each group (CKD, DM and HF) had a score below -4 but the difference from the control group did not reach statistical significance. Eight in 17 (50%) with liver cirrhosis and 1 in the control group (5%) had severe destabilization of reaction times, indicating CHE in the patients (p = 0.007). CKD had no effect on the result but DM and HF were associated with destabilization of reaction times in 6 and 8 patients respectively (p=0.03 and p=0.001). Patients with HF had results inferior to those in the cirrhosis group (mean index 2.0 vs. 1.5, p<0.04). Conclusion: Patients with CKD, DM and HF perform worse than age matched healthy participants in psychometric tests used to diagnose CHE and the CRT method confirmed the most. This means that a poor test result may be caused by CHE but also CKD, DM and especially HF and this should be taken in to consideration when interpreting psychometric tests used for diagnosing CHE in patients with liver cirrhosis and comorbid diseases.

![Fig 1: Top panel: PSE results from 18 healthy control participants, 18 patients with liver cirrhosis, 13 patients with heart failure, 15 patients with chronic kidney disease and 15 patients with deregulated diabetes type II. The tests result is abnormal when the sum score is < -4 (ticked line). The bottom panel shows CRTIndex results from the same patients. CRTIndex below 1.9 (ticked line) indicates CHE.](image-url)
Background and aim: The continuous reaction times (CRT) method describes arousal functions. Reaction time instability in a liver patient indicates covert hepatic encephalopathy (cHE). The effects of sleep deprivation are unknown although cirrhosis patients frequently suffer from sleep disorders. The aim of this study was to determine if sleep deprivation influences the CRT test. Method: Eighteen cirrhosis patients and 27 healthy persons were tested when rested and after one night’s sleep deprivation. The patients filled out validated sleep quality questionnaires. Results: Seven patients (38%) had unstable reaction times (CRTindex < 1.9) compatible with cHE. In these patients, the wakefulness improved reaction speed and reaction time stability (p=0.01), and in 3. CRTindex was normalized (CRTindex > 1.9). No change was found in reaction speed or stability in the remaining 11 patients. Seven patients (38%) reported poor sleep that was not related to their CRT tests before or after the sleep deprivation. In the healthy participants, the sleep deprivation destabilized (CRTindex < 1.9) and slowed reaction times by 11% (p < 0.0001) and in 7 persons (25%). Conclusion: In the patients with cHE reaction time stability improved or normalized by the acute sleep deprivation. The same intervention had no effect in patients with no cHE. There was no relation between reported sleep quality and reaction time results. This unexpected effect may be related to fast-acting changes in glutamatergic neurotransmission caused by wakefulness, as it is seen also in depressed patients. Importantly, in patients, sleep disturbances will not lead to ‘falsely’ slowed and unstable reaction times. In the healthy participants the acute sleep deprivation slowed and destabilized reaction times. Figure 1: CRTindex, before and after one night’s sleep deprivation, in 7 patients with liver cirrhosis and cHE (panel a), 11 patients with no cHE (panel b) and 27 healthy participants (panel c). The dotted line is the normal value cut-off of at 1.9 (lower abnormal).

Prevalence of Cognitive Deficit in Patients with Non-cirrhotic Portal Hypertension

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Background and aim: Hepatic encephalopathy (HE) is a well-known and frequent complication of liver cirrhosis but its prevalence in patients with non-cirrhotic portal hypertension is unknown. These patients have portal systemic shunts which may be responsible of Type B (Bypass) HE. The aim of the study is to describe the presence of cognitive deficit (overt and covert hepatic encephalopathy) in 34 patients with portal vein thrombosis (PVT) and 13 with idiopathic non-cirrhotic portal hypertension (INCPH).

Patients and Methods: The patients were evaluated by mini mental state to exclude dementia, and by a pool of standardized questions to detect and staging the presence of overt HE according to West Haven criteria. The psychometric hepatic encephalopathy score (PHES) which include 5 paper and pencil psychometric tests was used to detect the presence of covert HE. Each test was expressed as Z score adjusted for age and education of healthy Italian population. PHES ≤−4 was considered abnormal and diagnostic of covert HE. A group of 64 patients with compensated cirrhosis (Child-Pugh A) were submitted to the same evaluation and used as controls.

Results: Mean age was significantly (p=0.0001) higher in cirrhotic patients. At the evaluation, signs of portal hypertension (esophageal varices, variceal bleeding, ascites) were equally present in the three groups. No signs of overt HE were detected at the time of evaluation but prior overt HE episodes occurred more frequently (p=0.04) in cirrhotic patients than in patients with PVT or INCPH. Psychometric performance was poorer in cirrhotic patients but the prevalence of covert HE according to PHES was non significantly different in the three groups (Table1).

Conclusion: The psychometric performance of patients with non-cirrhotic portal hypertension is better than that of compensated cirrhotic patients but cognitive alterations are not infrequent: one patients with experienced a episode of overt HE and 20% of them have a cognitive deficit if tested (covert HE). The clinical consequence of this observation deserves further investigations.

A low-cost, user-friendly EEG recording set for HE assessment: a proof of concept, preliminary study

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Background: Electroencephalography (EEG) is useful to objectively diagnose/grade HE across its spectrum of severity. In addition, the EEG has recently been shown to improve the prognostic value of MELD. However, it requires expensive equipment, it is time-consuming, and hepatogastroenterologists are generally unfamiliar with its use/interpretation. Recent technological advances have lead to the development of low-cost, user-friendly EEG recording systems, allowing EEG acquisition in limited neurophysiological experience settings.

Aim: To assess the relationship between EEG parameters obtained from a Standard-EEG system and from a commercial, low-cost wireless headset (Light-EEG) in a group of well-characterized patients with cirrhosis.

Methods: Forty patients with cirrhosis (32 males; 60±10 years) underwent EEG recording with both types of equipment, within 20 minutes. Standard spectral EEG parameters [i.e. Mean Dominant Frequency (MDF) and the relative power of the theta band (theta%)] were obtained from a bi-parietal derivation on both EEGs. Spearman’s rank correlation coefficient and the Bland-Altman method were utilized to evaluate correlation and agreement, respectively, between measures obtained from the two EEG recording tools. In addition, correlations between clinical parameters (MELD and ammonia) and Light-EEG spectral parameters were computed.
ABSTRACTS

Results: Strong correlations were observed between spectral parameters obtained from the two EEG systems (MDF= 0.52; ps < 0.001; \(\text{r}^2\text{theta}%= 0.83; p< 0.0001\)). Bland-Altman analysis indicated that spectral parameters obtained from the Standard and Light-EEG systems were comparable, with clinically acceptable ranges of oscillation and no systematic variation of the differences across the range of measurement. Spectral parameters obtained from the Light-EEG correlated significantly with both the MELD score (MDF= -0.49, p=0.036, \(\text{r}^2\text{theta}%=0.61, p=0.007\)) and fasting, venous ammonia levels (MDF=-0.47, p=0.018; \(\text{r}^2\text{theta}%=0.47, p=0.016\)).

Conclusions: Reliable EEG parameters for purposes of HE evaluation can be obtained from a commercial wireless headset. This may lead to more widespread use of this operator-patient-independent tool for HE assessment in routine hepatological practice and in the research setting.

29 A SIMPLE OPERATIVE CRITERION TO ASSESS PATIENTS WITH GRADE 1 HEPATIC ENCEPHALOPATHY: THE ‘ANIMAL NAMING TEST’

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Background and aim. Grade 1 HE (according to West Haven Criteria) is poorly discernible and does not have an operative definition. The Animal Naming Test (ANT), i.e. the enumeration of the maximum number of animals in 60 sec, might be a useful tool for routine clinical assessment. Therefore, we performed a study to evaluate the usefulness of ANT in the detection of patients with grade 1 HE.

Methods. 208 healthy subjects stratified by decade of age (mean age 54 ± 19 y.) and, where available, education (education 10±5 y.), and 208 consecutive cirrhotic patients: 86 unimpaired (Psychometric Hepatic Encephalopathy Score –PHES≥ -4) (age 58±14 y., education 10±4 y., MELD score 12±5), 83 with MHE (PHES=4) (age 63±12 y., education 8±4 y., MELD score 14±6) and 39 with grade 1 HE (evaluation of an expert hepatologist) (age 62±12 y., education 8±4 y., MELD score 14±5) underwent the ANT. The sensitivity/ specificity of ANT for an operative diagnosis of grade 1 HE was tested by ROC analysis. In a subgroup of patients, the relationship between ANT and EEG (n=147) and CFF (n=95) was evaluated.

Results. In controls ANT was found to be correlated with education and age (p<0.001). However, in patients a simpler model was obtained showing that ANT≤10 (Psychometric Hepatic Encephalopathy Score 0% and 0% respectively; 85% and 85% respectively) for grade 1 HE in patients with educational level ≤12 years, and ANT≤12.5% (CIF5%: 28-85) sensitivity and 71% (CIF5%: 59-81) specificity for grade 1 HE in patients with educational level > 12 years. In the latter, ANT≤10 had 33% (CIF5%: 10-65) sensitivity and 82% (CIF5%: 72-90) specificity. An abnormal score of ANT based on the above thresholds was associated with a higher risk of EEG alteration (OR=2.7 CIF95%=1.3-5.8) and a trend for a higher of CFF (OR=2.0 CIF95%=0.7-5.6).

Conclusions. ANT can be used as a simple operative criterion to improve the clinical assessment of HE.

30 Cognitive Reserve in the assessment of Minimal Hepatic Encephalopathy

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Introduction: The so-called Cognitive Reserve (CR) is the ability to actively cope with brain dysfunction by recruitment of compensating cognitive resources. CR may fully or partly explain the absence/limited cognitive dysfunction despite proven brain damage. Previous research has shown that life-long cognitive stimulation and training (i.e. years of education, working, sport and leisure time activities carried out) can be utilized to estimate CR. The aim of this study was to evaluate the effect of CR in the assessment of Minimal Hepatic Encephalopathy (MHE) in patients with cirrhosis.

Method: Eighty-two outpatients with cirrhosis (60 males; mean age: 60 ± 11 years; mean educational level: 10 ± 4 years) underwent: 1) the Portocentric Systemic Hepatic Encephalopathy Score (PHES), which was scored according to age- and education-adjusted Italian norms; 2) the Cognitive Reserve Index questionnaire (CRIq); 3) awake EEG recording: this was analyzed spectrally and the Mean Dominant Frequency (MDF) of the bi-parametrical P3-P4 was utilized as a summary EEG index. Finally, the ratio PHES/MDF was computed and considered as an index of cognitive functioning, corrected by individual neuropsychological status at the time of the evaluation.

Results: One-way ANOVA showed that the lower the CRiq, the lower the PHES, regardless of EEG features (F(3,78)=3.19, p<.05). Spearman’s rank correlation analysis showed that the CRiq was correlated to PHES both in patients with and in those without MHE (r=0.35, p<0.05; r=0.30, p<0.05).

Conclusions: Cognitive performance of patients with cirrhosis, both with and without MHE, is related to and predicted by Cognitive Reserve. In addition, Cognitive Reserve may explain, at least to some extent, the mismatch between cognitive and EEG performance which is often observed in these patients.

31 The Utility of an Automated Segmentation Tool to Measure Volumes of Sub-Cortical Structures in Hepatic Encephalopathy

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Abstract

Background: Technology to measure small changes in brain volume on clinical MRI scans has developed rapidly over recent years with the ability to assess individual sub-cortical brain structures reliably and accurately.

Objectives: (i) To measure subcortical brain volumes on MRI in hepatic encephalopathy (HE) using the new automated FMRIB Integrated Registration & Segmentation Tool (FIRST) and (ii) to assess volume changes with treatment intervention.

Method: 247 control MRIs (age range 18-85) were collated from publically available datasets. The subcortical brain structure volumes were compared with age. Then, a group comparison of 7 minimal HE (MHE) patients’ MRIs, were compared with 60 controls within the appropriate HE age range. Volume changes in the putamen and accumbens volumes were assessed 12 weeks apart. All 7 patients were given placebo or L-Ontamine L-Aspartate (LOLA) after visit 1.

Results: A trend to decreasing subcortical brain volumes was seen with increasing severity. On comparison of the controls and MHE patients, a significant reduction in the left putamen (p=0.009) and right accumbens (p=0.048) volume was measured in the MHE patients. On closer observation of the putamen and accumbens, the two of patients who were given LOLA displayed an improvement in brain volume after the 12 week treatment interval.

Conclusions: This study has shown the utility of this new imaging registration technique in assessing very small sub-cortical structures. A significant change in subcortical brain structures in MHE patients was seen, but ongoing work needs to augment numbers to validate the technique.
ABSTRACTS

Recalification Index was 0.3 (p=0.04). Both models (3 variables AUC=0.77; CI: 0.56 – 0.91) (2 variables AUC=0.76; CI: 0.58 – 0.94) were validated by the independent series of patients in Padua.

Table 1. Demographic and clinical characteristics and prevalence of previous overt HE and minimal HE according to PHES in the patients included in the observation group (Rome).

| Number of Pts. | 216 |
| Age (years) | 63 ± 12 |
| Sex (M/F) | 145/71 |
| Education (years) | 9.2 ± 4.2 |
| Aetiology of cirrhosis (viral/alcohol/other) | 146/51/19 |
| Child Class (A/B/C) | 105/85/26 |
| MELD score | 12.6 ± 5.3 |
| Presence of esophageal varices n (%) | 111 (52%) |
| Ascorbs n (%) | 98 (45%) |
| TIPS n (%) | 15 (6%) |
| Evidence of P/C shunt n (%) | 35 (16%) |
| Albumin (g/dL) | 3.4 ± 0.8 |
| Sodium (mEq/L) | 136.6 ± 4.6 |
| Bilirubin (mg/dL) | 2.0 ± 1.16 |
| Creatinine (mg/dL) | 0.8 ± 0.54 |
| INR | 1.4 ± 0.34 |
| Patients with grade II overt HE; n (%) | 48 (22%) |
| Patients with minimal HE according to PHES < ≤ n (%) | 96 (44%) |
| PHES (score) | -3.5 ± 3.5 |
| TMT-A (sec.) | 66.4 ± 36.6 |
| TMT-B (sec.) | 135.2 ± 78 |
| DS (n.) | 21.5 ± 10.3 |
| SDT (sec.) | 76 ± 40.3 |
| LTT (sec.) | 99.7 ± 38.7 |
| LTT (err.) | 61.3 ± 74.4 |

Mean ± SD

*p patients didn’t undergo upper endoscopy

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Title: Bispectral Index for Diagnosis and Assessment of Response in Overt and Minimal Hepatic Encephalopathy Before and After Treatment in Patients with Cirrhosis

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Background/Aims: Severity of hepatic encephalopathy (HE) is graded using West Haven criteria and psychometric tests. There is no gold standard for grading of HE and monitoring its progression. We evaluated utility of bispectral index (BIS) to diagnose minimal HE (MHE) and overt HE, grade HE and monitor improvement or worsening of overt HE and MHE with treatment.

Methods: 200 patients of cirrhosis (50 each of HE grade I, II, III and IV, 60 of MHE and 20 without HE/MHE were enrolled. Assessment of grades of HE was done by West Haven criteria and MHE by psychometric tests. BIS was performed at baseline in patients with overt HE, MHE, cirrhotics without HE/ MHE and 20 healthy controls. BIS was repeated after lactulose therapy of one week in overt HE and 3 months in MHE. Significance between different groups was calculated by ANOVA, Post-hoc analysis and cut off values determined by ROC curves.

Results: BIS values were significantly different in patients with different grades of HE and MHE; 79.5±4.2, 67.5±4.3, 56.4±3.5, 44.8±3.9 and 85.4±3.4 respectively for grade I, II, III, IV HE and MHE, but similar (92.6±3.7 vs 93.7±5.2) in cirrhotics without HE/MHE and healthy controls. BIS cut off values for MHE, HE grade I, II, III and IV were 90.5, 77.5, 70.5, 60.5 and 50.5 respectively. Mean BIS scores after treatment were 79.7±4.0, 67.5±4.4, 56.1±3.4, 43.7±3.4, 82.4±5.6 and 90.1±4.8 respectively for patients of overt HE grade I, II, III, IV, MHE and without HE. Changes in BIS values after treatment corresponded to BIS cut off scores for grades of HE and MHE.

Conclusions: Bispectral index is useful measure for diagnosing minimal and overt hepatic encephalopathy, grading hepatic encephalopathy and monitoring improvement or worsening of minimal and overt hepatic encephalopathy with treatment in patients with cirrhosis.

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MAY A BETTER NUTRITIONAL STATUS REDUCE THE OCCURRENCE OF HEPATIC ENCEPHALOPATHY IN CIRRHOTIC PATIENTS WITH SEPSIS?

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Background and aims: Malnutrition represents an important burden in cirrhosis and protein malnutrition (PM) has been associated to increased rate of hepatic encephalopathy (HE). We aimed to analyse if nutritional status could influence the rate of HE in cirrhotic patients with sepsis.

Materials and methods: Consecutive hospitalised cirrhots with sepsis were enrolled in the study. PM was diagnosed if Mid-Arm-Muscle Circumference was <5th percentile. HE was clinically diagnosed and staged according to West-Heaven criteria.

Results: Seventy-four patients (71% males; median age 64yrs; median Child-Pugh 9 and MELD 14) were enrolled. PM was diagnosed in 43% of patients. Severity of liver disease and characteristics of infection were not different in patients with and without PM. A stratified analysis according to the Child-Pugh class, was performed. In patients with Child-Pugh class C (29 patients) HE during sepsis in patients with versus those without PM was similar (grade I-II 25% vs 30%; grade III-IV 23% vs 19%; p=0.6); in-hospital mortality was also not significantly different (46% vs 44% respectively; p=0.9). In Child-Pugh A-B patients (45 patients), HE was more frequent in patients with vs those without PM (grade I-II 21% vs 8%, grade III-IV 21% vs 0%, p=0.01); in-hospital mortality was also significantly higher in patients with versus without PM (50% vs 16%; p=0.01).

Conclusion: Our study shows that PM represents a risk factor for HE and mortality in cirrhotic patients with sepsis, which worsens the outcome of patients with mild-moderate cirrhosis. In Child-Pugh class A-B patients with PM the prognosis of sepis need to be considered as severe as in patients with a more advanced liver disease.

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LONG-TERM-FOLLOW UP REVEALED “RAPID CYCLING” OF COGNITIVE FUNCTION IN PATIENTS WITH HCV-ENCEPHALOPATHY

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Background and Aim: Hepatitis C virus (HCV) infection is associated with chronic fatigue, reduced quality of life and altered cognitive function in about 50 % of the patients. Affected patients complain about sudden drops in their alertness and progression of symptoms over time. This study aims to analyze cognitive function of HCV-infected patients in a long-term follow-up.

Methods: 25 HCV patients (age 50.1, SD 6.9) who had been examined for the first time between 1999 and 2006 (T0) underwent follow-up examinations in 2008 (=T1; age 55.5, SD 8.1) and 2012-2013 (=T2; age 60.2, SD 7.7). Five healthy controls who had been examined at T0 could be re-examined at T2 as well. Patients and controls filled in self-assessment questionnaires for fatigue, mood and quality of life, and in addition underwent a neuropsychological test battery including attention and memory tests. Blood samples were collected for calculation of the APRI (aspartate-aminotransferase -to-platelet-ratio-index) score. The Friedman test was used for statistical analysis.

Results: The APRI scores did not significantly change over time. There were also no significant differences of the median fatigue, quality of life, depression and anxiety scores. Significant deterioration, however, was observed for the PSE Symptom Test result (p<0.001) from T0 to T1 and T2, for the complex copy task but not the retrieval task between T1 and T2 (p<0.001) and for the recognition of figures in the word-figure-memory test between T0 and T1 (p< 0.05). A significant improvement of the latter was observed from T1 to T2 (p<0.002). A significant improvement was also observed for the median percentile of the number of items processed in the cancelling d test minus errors (p<0.001) between T2 and T3. Detailed analysis showed gross variations of the individual results not only with regard to the absence or presence of changes and their direction, but also with regard to the level of performance within a single session. In contrast the test results remained stable in the controls.

Conclusions: Subjective symptoms as well as objective cognitive dysfunction varied significantly over time in both directions, not only between but even within the different assessment sessions - sort of “rapid cycling”. This finding points to a significant alteration of the patients’ alertness system, and needs to be clarified by further neurophysiological, neuropathological and imaging studies.
Regional Cerebral Water Contents in Hepatic Encephalopathy measured by MRI.

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Background & Aims: The pathophysiology of hepatic encephalopathy (HE) may involve cellular cerebral edema due to osmotic swelling caused by glutamine accumulation. However, it has only been studied in patients with overt HE or by indirect methods.

Methods: We measured the absolute brain water contents with anatomical resolution by MRI in 7 cirrhosis patients during an episode of overt HE type C. Six patients with cirrhosis and no history of HE and 12 healthy age matched control subjects were also scanned. Two patients were rescanned after recovery from HE. The images were normalized to standard space and the image analysis used volumes of interest from a probabilistic brain atlas.

Results: The average whole brain water contents in both patients with HE and cirrhosis patients who never suffered from HE was 85 ± 0.03 vs. 83 ± 0.02 (P = 0.14) in the healthy controls (Figure 1) Temporal lobe water was 86 ± 0.03, 85 ± 0.04, and 82 ± 0.04 in HE, cirrhosis, and controls, respectively. The corresponding cerebellum water was 86 ± 0.02, 84 ± 0.02, and 82 ± 0.02. The frontal lobe water was 84 % ± 0.03 in both HE and cirrhosis patients and 82 % ± 0.02 in the controls (P <0.05, all regions HE vs. controls). The brain water contents of the two patients with overt HE fell after recovery by 0.9 % and 1.3%.

Conclusions: Our data quantitatively demonstrate low-grade cerebral edema in several brain regions in patients with overt HE and suggest that it may be reversible with recovery from HE. Cirrhosis patients without HE may also have slightly increased brain water.

Background: Minimal hepatic encephalopathy (MHE) is the first stage of the spectrum of hepatic encephalopathy (HE) and is only detected by psychometric tests. Psychometric hepatic encephalopathy score (PHES) has been confirmed to be useful to diagnose MHE. Recently, Kircheis et al. (Gastroenterology 2014; 146: 961–969) have proposed a modified PHES to detect MHE, but its relationship with overt HE was not assessed.

Aim: To validate the modified PHES to diagnose MHE and check its usefulness to predict the developing of overt HE.

Methods: We included prospectively 117 cirrhotic patients. MHE was assessed by critical flicker frequency (CFF) < 39 Hz. Conventional PHES consisted of the Number Connection Test A (NCT-A) and Number Connection Test B (NCT-B). Digit Symbol Test (DST), Line Tracing Test (LTT, time and errors), and Serial Dotting Test (SDOT). Modified PHES was calculated without SDOT, according to the original article mentioned in Background. Conventional PHES was defined as pathological with < 4 points and modified PHES with ≤ 1 point, according to the original article by Kircheis et al.

Results: Out of 117 patients, 71.8% (84/117) were males and mean age of 57.5±11.1 years old. Liver function was assessed by Child-Pugh (7.2±2.4) and MELD score (11.9±5.9). Patients were followed-up during 10.6±4.2 months. During the study, 19.3% (21/109) of patients suffered overt HE and 12.7% (14/110) died. MHE was diagnosed in 47% (55/117), according to CFF < 39 Hz. Conventional PHES was associated with MHE (75.7% (28/37) vs 33.8% (27/80); p < 0.0001), as well as modified PHES (58.6% (41/70) vs 30.8% (12/39); p=0.005). However, conventional PHES was associated with overt HE (40% (14/35) vs 9.5% (7/74); logRank 11.54; p=0.001), but not modified PHES (23.8% (15/63) vs 7.9% (3/38); logRank 2.67; p=0.103). Adjusting by age, and previous event of overt HE, conventional PHES was independent associated with overt HE [HR 4.04 (95% CI 1.55-10.56); p=0.004], together with MELD score [HR 1.14 (95% CI 1.07-1.22); p<0.0001]. However, modified PHES was not associated with overt HE including the same variables in multivariate analysis.

Conclusion: Modified PHES was associated with minimal hepatic encephalopathy, as Kircheis et al. observed. However, conventional PHES was related to predict the developing of overt hepatic encephalopathy, while modified PHES was not able to predict it.

Title: Development of a predictive model identifying intracranial hypertension in patients with acute liver failure.

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Background and aims: Acute liver failure (ALF) is a life threatening condition that in combination with persistent hyperammonaemia carries a high risk of brain oedema and subsequently intracranial hypertension (ICH). The pathophysiology involved is believed to include cerebral glutamine accumulation and a compromised oxidative metabolism. In this study we aimed to construct a predictive model in order to identify patients developing ICH.

Material and methods: 45 patients (26 females) with ALF and persistent hyperammonaemia (>150 µM for 24 hours) or clinical signs of ICH were included over a ten year period. Cerebral microdialysis and monitoring of intracranial pressure (ICP) was initiated while the patients were mechanically ventilated and sedated. The concentration of lactate, pyruvate and glutamine was measured in the microdialysate. Baseline values of ICP, arterial lactate and ammonia were measured upon inclusion. Furthermore the need for renal replacement therapy and/or vasopressors was registered. ICH was defined as ICP>20 mmHg for more than one hour. We modelled a highly flexible classifier using a support vector machine with radial kernel and evaluated the prediction error rate of ICH/non-ICH with five-fold cross validation.

Results: 21 patients (47%) developed ICH (30-108) hours (median (range)) after inclusion and was the cause of death in ten of those patients. The baseline arterial ammonia was 186 (60-340) µM, the cerebral lactate to pyruvate ratio 29 (9.1-173) and glutamine 3.2 (0.2-7.3) mM. By comparison of patients with and without ICH only glutamine was significantly different (3.7 ± 2.7 mM respectively, p<0.05). Building a model on clinical variables not including ICP and microdialysate gave a prediction error rate of 36% and adding baseline ICP and microdialysate measurements did not improve the performance.

Conclusion: ICH is a frequent complication in this subgroup of ALF patients and is associated with elevated extracellular glutamine in the brain as previously reported. The median lactate to pyruvate ratio was close to normal levels. Building a predictive model did not allow us to classify patients to ICH/ non-ICH correctly in more than 64 % of the cases. This most likely reflects a mismatch between a complex psychophysical and limited amounts of data from this rare type of patients.

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Title: The Development and Validation of a New Psychometric Battery for the Detection of Minimal Hepatic Encephalopathy

Authors

N.A. Cook, Y Pasha, M.M.E Crossev, S.D Taylor-Robinson

Background: Psychometric testing is used in clinical practice to identify patients with cirrhosis who have developed hepatic encephalopathy (HE). Minimal hepatic encephalopathy (mHE) is difficult to diagnose. The only validated battery consists of 5 paper-and-pencil tests. A modern, easy to use, computer battery maybe helpful for diagnosis, given that mHE has an impact on both patient quality of life, driving safety and machine operation (with societal consequences).

Aim: The study compares CogstateTM computer battery testing with a validated Hepatic Encephalopathy Score (PHES) tests, with a view to facilitating early diagnosis.

Methods: This was a prospective study of 27 patients with histologically proven cirrhosis. Analysis of psychometric testing was performed using accuracy of task performance and speed of completion as primary variables to create a correlation matrix. The correlation matrices were transformed into heatmaps. A stepwise linear regression analysis was performed with backward elimination, using analysis of covariance (ANOVA).

Results Strong correlations were found between the International Shopping List Delayed Recall of CogstateTM and the PHES Digit Symbol Test. The PHES Serial Dotting test did not appear to be discriminatory. The Shopping List Tasks were the only tasks that consistently had p values of <0.05 in the linear regression analysis.

Conclusion: Subtests of the CogstateTM battery correlated very strongly with the Digit Symbol (PHES) in discriminating severity of HE. Findings indicate that some of the PHES battery without Serial Dotting and including the International Shopping List tasks of CogstateTM may be more discriminant in clinical practical.

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Health Related Quality of Life of Patients with Previous Overt Hepatic Encephalopathy Compared to Age and Sex Matched Cirrhotic Controls

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Introduction Hepatic Encephalopathy (HE) is known to adversely affect Health Related Quality of Life (HRQOL). However, there is a lack of consensus on the pattern of impairment and the best tools to assess HRQOL in HE.
41: Brain metabolism in Minimal hepatic encephalopathy assessed by 3.0tesla MRI

Methods: We examined 14 patients with liver cirrhosis. (9 male, 5 female, mean age 59.3 y.o.) Patients were evaluated neuropsychiatric symptoms by neuropsychiatric test such as Digit symbol test (DST), Block design test (BDT), Number connection test A,B (NCT A,B). We diagnosed as MHE who had 2 or more abnormalities in these tests. All the patients were examined with MRI imaging T1-SPGR, T2, structural and Magnetic Resonance Spectroscopy (MRS) by 3T MRI. We compared MHE group (3 patients), and Non-MHE group (11 patients).

Results: Compared to MHE and Non MHE group, Glutamine level of MHE group (Gln/Cr) 2.67±0.46 (<0.001), Glutamate level of MHE group (Glu/Cr) 1.37±0.15 was significantly lower than Non MHE group (Glu/Cr) 1.73±0.13. Also, Myoinositol level of MHE (Mio/Cr) 0.28±0.04 was significant lower than Non MHE (Mio/Cr) 0.38±0.16 (<0.001). In MRI imaging, most of the patients had high intensity in pallidum in T1-SPGR. However, there was no significant structure difference between two groups. There was no significant difference between blood NH3 level of MHE group and Non MHE group.

Conclusions: These results suggest that brain Glutamine and Myoinositol metabolism have been changed in MHE. These changes may contribute to mechanism of pathogenesis in MHE. High-resolution 3T MRS might be useful for diagnosis of MHE.

42: The performance of the EEG in hepatic encephalopathy is enhanced by use of multivariable threshold selection of diagnostic spectral variables Clive D Jackson,1 Meike Heeren,2 Gerrt M Große,2 Anita Tryc,3 Henning Pfugrad,4 Richard W Morris,1 Karen Weissenborn,2 Marsha Y Morgan4

Methods: Patients were selected from an ongoing longitudinal study of HRQOL in cirrhosis. Patients with a previous history of Overt HE (OHE) were matched by age and sex to cirrhotic patients without previous OHE. Baseline clinical data and results from the following HRQOL measures were available: SF-36, CLDQ, PROMIS-HAQ and the general domains of PBC-40.

Results: 22 patients with a previous history of OHE could be matched to non-OHE cirrhotic controls (total n=44). Median MELD scores were 8 in the OHE group and 10 in the non-OHE group (p=0.07). Patients in the OHE group had significantly prolonged Number Connection Test (NCT) completion times (56 sec vs 45 sec, p=0.01). SF-36 data showed that both groups had considerably impaired HRQOL when compared to normative data, affecting both physical and mental aspects, but no significant differences between the two groups were seen in any of the SF-36 scores. There was evidence of more severe emotional impact in the OHE group with significantly poorer CLDQ-Emotional Function (EF) scores (4.3 vs 6.0, p=0.46). Furthermore, differences in PBC-40 Emotional domain scores were nearing significance (10.0 vs 3.5, p=0.085). Social functioning was more greatly impaired in the OHE group with significantly poorer PBC-40 social domain scores (37.5 vs 27.0, p=0.028) and differences in the SF-36 Social Functioning (SF) domain scores which were nearing significance (32.3 vs 39.8, p=0.106). The PBC-40 cognitive domain also responded to OHE (17.0 vs 13.0, p=0.033).

Conclusion: Although it is clear that HE adversely affects HRQOL, some widely used HRQOL measures may not accurately reflect this. The general domains of PBC-40 performed well in this small study and may merit further evaluation in larger studies of HE.

43: Sleep-wake Disturbances in Hepatic Encephalopathy: Use of a Sleep-deprived Protocol

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Introduction: Sleep disorders are common in patients with cirrhosis; both excessive daytime somnolence (EDS) and night-sleep disturbance are reported. There is general agreement that the presence of EDS is related to the presence of hepatic encephalopathy (HE), but the relationship between EDS severity and HE remains controversial. Overnight sleep studies are difficult to undertake in these patients; use of sleep-deprived protocols may help clarify this situation.

Aim: To determine the relationship between sleep-wake abnormalities and the presence and degree of HE in patients with cirrhosis utilizing additional data from a sleep-deprived protocol.

Methods: Thirty-eight patients with cirrhosis (61% men: mean [range] age 60 [37-85] yr), classified as either neuropsychiatrically unimpaired (n=20), or as having minimal (n=6) or overt HE (n=12), and 50 healthy controls (52% men: age 53 [41-65] yr), were recruited. Sleep-wake behaviour was assessed using the Epworth Sleepiness Scale (ESS); and the Pittsburgh Sleep Quality Index (PSQI). Subjects’ ability to fall asleep in a dark, quiet room, during daytime, was assessed using a strict sleep-deprivation protocol; 24 patients and 26 controls were compliant. Attainment of a minimum of 2 min of Stage II sleep within the first 40 min of EEG recording was classified as ‘able to sleep’.

Results: Patients receiving significantly more day-time sleepiness than controls. Mean ±SD ESS score [±%11] increased with the degree of neuropsychiatric impairment: (unimpaired 4.4±2.6% vs overt HE 12.6±6.1% [70%]; p<0.001). ESS scores correlated significantly with the awake EEG theta % (r=0.45, p<0.02). Patients also reported significantly worse sleep quality than controls. Mean PSQI scores [%±5] increased with the degree of neuropsychiatric impairment (unimpaired 5.4±3.5% [37%] vs overt HE 9.5±4.9% [80%]; p<0.05). PSQI scores showed significant inverse correlation with PHES scores (r=-0.58, p<0.01). ESS and PSQI scores correlated significantly (r=-0.49, p<0.01). ESS scores and PSQI scores were higher in the patients able to fall asleep during the day; patients with overt HE were more likely to sleep (Table).
## ABSTRACTS

### 44 Homeostatic Sleep Disturbances & Hepatic Encephalopathy in Patients with Cirrhosis

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**Introduction:** Patients with cirrhosis have significantly disturbed sleep-wake behaviour. However, the cause of this disturbance is uncertain and its relationship to the presence of hepatic encephalopathy (HE) is unclear. Sleep is regulated by circadian and homeostatic processes. Cirrhotic abnormalities, while present in these patients, do not correlate with the observed disturbances in sleep-wake behaviour. Homeostatic sleep mechanisms are difficult to access however sleep-spindles, which are a feature of the sleep electroencephalogram (EEG), are generated by thalamo-cortical oscillatory networks and act as a surrogate for homeostatic sleep processes. These same oscillatory networks have been implicated in the pathogenesis of HE.

**Aim:** To examine the sleep EEG in patients with cirrhosis and to relate any changes observed to sleep behaviour and the presence and degree of HE.

**Methods:** Thirty-eight patients with cirrhosis (61% men; mean [range] age 60 [37-85] yr), classified as either neuropsychiatrically unimpaired (n=20), or minimal HE (n=3) or overt HE (n=15), were recruited. Sleep-wake behaviour was assessed using validated questionnaires. All subjects returned within 3 days of their initial assessment for a sleep-deprived EEG which mandated 4 hours of sleep deprivation from the night-sleep episode preceding the sleep EEG; all sleep EEGs were conducted between 13:00 and 16:00 to minimise the confounding effect of the circadian phase. Twenty-four (63%) patients and 50 healthy controls (52% men; age 53 [41-65] yr), were recruited. Sleep-wake behaviour was assessed using validated questionnaires. All subjects returned within 3 days of their initial assessment for a sleep-deprived EEG which mandated 4 hours of sleep deprivation from the night-sleep episode preceding the sleep EEG; all sleep EEGs were conducted between 13:00 and 16:00 to minimise the confounding effect of the circadian phase. Twenty-four (63%) patients and 50 healthy controls (52% men; age 53 [41-65] yr), were recruited. Sleep-wake behaviour was assessed using validated questionnaires. All subjects returned within 3 days of their initial assessment for a sleep-deprived EEG which mandated 4 hours of sleep deprivation from the night-sleep episode preceding the sleep EEG; all sleep EEGs were conducted between 13:00 and 16:00 to minimise the confounding effect of the circadian phase. Twenty-four (63%) patients and 50 healthy controls (52% men; age 53 [41-65] yr), were recruited.

**Results:** Sleep-disturbances were common in the patients and were significantly more frequent in the patients with overt HE. No visually identifiable differences were observed in sleep EEGs between controls and neuropsychiatrically impaired patients. However, easily discernible differences were observed in the EEGs from patients with overt HE (Figure). The mean±1SD P-SRAT was significantly greater in the patients with overt HE than in the controls (59.2±3.8 vs. 52.4±2.8; p=0.001) and unimpaired patients (59.2±3.8 vs. 53.9±1.5; p<0.005) indicating disturbed spindle formation and occurrence.

**Conclusion:** Significant abnormalities were seen in the sleep EEGs in patients with overt HE all of whom reported disturbed sleep-wake behaviour. Disruption of cerebral oscillatory networks may underlie both the sleep disturbances and the neuropsychiatric abnormalities observed in patients with cirrhosis.

Legend to Figure:
A: Sleep EEG in a neuropsychiatrically impaired patient with cirrhosis showing well defined spindles (arrows)
B: Sleep EEG in a patient with overt HE; waveforms are of high amplitude (box) and spindles poorly formed

### 46 RIFAXIMIN IS EFFICACIOUS IN THE TREATMENT OF CHRONIC OVERT HEPATIC ENCEPHALOPATHY: A UK LIVER MULTI-CENTRE EXPERIENCE

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**Introduction:** Rifaximin is a non-absorbable antibiotic increasingly being used for the secondary prevention of recurrent overt hepatic encephalopathy (HE) in the UK. The therapeutic mechanism of rifaximin has yet to be elucidated, with reduction in gut ammonia production postulated. We undertook a UK multi-centre retrospective audit of patients receiving rifaximin therapy for HE in 4 hospitals, two of which are liver transplant units, with the aim of assessing tolerability, impact on HE/liver disease severity and hospitalisation rates.

**Methods:** Patient demographics, concurrent therapy, Child Pugh, MELD, UKELD and number of hospital admissions were collected 3 months prior to initiation of rifaximin therapy and then 3 months following treatment.

**Results:** 170 patients were identified (mean age 57 yrs±12, 68% male) over the period 05/2010–03/2013. Three month post treatment outcome data were available for 73 patients (43%); 53 patients (31%) died during the 3 month follow up period. Average duration of treatment was 79±121 days, with therapy

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**Legend to Figure:**
A: Sleep EEG in a neuropsychiatrically impaired patient with cirrhosis showing well defined spindles (arrows)
B: Sleep EEG in a patient with overt HE; waveforms are of high amplitude (box) and spindles poorly formed
Conclusion: Our UK multi-centre experience is that rifaximin is well-tolerated and an efficacious treatment for the secondary prevention of HE. Rifaximin significantly reduced both hospital re-admission rates after 3 months treatment, impacting significantly on the NHS resource burden of HE, and reduced overall liver disease severity raising the possibility that its therapeutic effect may extend beyond reducing gut ammonia production.


47 Cerebral white matter lesions in patients with liver cirrhosis - causative for hepatic encephalopathy or bystanders?

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Background & Aims: Focal white matter lesions mimicking microvascular lesions were connected to the development of hepatic encephalopathy (HE) in patients with liver cirrhosis. This study aims to assess the relationship between cerebrovascular risk factors and the prevalence and extent of these lesions in patients with cirrhosis, as well as their impact upon cognitive function.

Methods: 55 cirrhotic patients underwent neurological examination, psychometric testing and magnetic resonance imaging. T2-weighted images were reviewed for white matter lesions by a neuroradiologist and a neurologist, independently. Patients were allocated into 3 groups: 1) no or <5, 2) 6-15 and 3) more than 15 lesions. Allocation was confirmed by a senior neuroradiologist blinded for the clinical data. The patient groups were compared concerning age, underlying liver disease, mortality, MELD Score, history of HE, treatment for HE, cerebrovascular risk factors and psychometric test results. Regression analysis was performed to identify risk factors for the presence and extent of white matter lesions.

Results: Patient groups 2 and 3 were older and showed worse results in the psychometric tests than group 1 (p<0.05). Correlation analyses showed a significant relationship between the number of white matter lesions and the grade of HE (p<0.001) and cognitive function (p<0.05), but no interrelationship between the lesions and cerebrovascular risk factors or other factors tested.

Conclusions: Focal white matter lesions in patients with liver cirrhosis do not represent arteriosclerotic microangiopathy but are related to the pathology of HE. Further studies are needed to clarify the mechanisms behind in detail.

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48 Apraxia in cirrhotic patients, influence of hepatic encephalopathy.


Background: Patients with hepatic encephalopathy (HE) display mental and motor changes. Many cirrhotic patients show some type of neuropsychological abnormalities. However, apraxia disorders have not been well characterized in them.

Aim: Assess the presence of apraxia in compensate and decompensate cirrhotic patients and the influence of previous hepatic encephalopathy.

Methods: We designed a neuropsychological test battery to assess the praxis that included: a subset of praxis of the 2nd edition of the Boston’s Aphasia battery (graphomotor and non graphomotor sequences, reciprocal coordination, non symbolic intransitive praxis) and Barcelona Test (buccofacial praxis, gestural intransitive praxis and transitive praxis to command and imitation). In addition to this battery, we also used other neuropsychological test to screen general executive functions (FAB), speed of information processing (Symbol digit oral version, SDO), complex cognitive motor control (Grooved Pegboard, GP), and minimal hepatic encephalopathy (PHES). This battery was administered to: a) 101 cirrhotic patients; 28 compensated and 73 decompensated (27 with previous hepatic encephalopathy) and b) 101 healthy controls matched by sex, age and years of education. At the evaluation, none of the patients had signs of hepatic encephalopathy.

Results: Compared to healthy subjects, cirrhotic patients showed significant impairment in most praxis and related test: graphomotor sequences (p<0.03), non graphomotor s. (p<0.001), coordination (p<0.001), non symbolic intransitive praxis (p<0.005), gestural intransitive praxis (p<0.05), transitive praxis with all the body to command (p<0.002), FAB (P<0.001), GP (p<0.001) and SDO (p<0.001). There were no significant differences between compensated and decompensate cirrhotic patients. In decompensated cirrhosis, patients with previous encephalopathy had significant impairment in the most of neuropsychological tests (p<0.005) than those without previous encephalopathy. Severity of apraxia was correlated with age, PHES score and less extended with severity of liver cirrhosis (MELD and Child-Pugh scores).

Conclusions: Apraxia disorders are present in patients with hepatic cirrhosis. Cirrhotic patients had more apraxia disorders than controls, but severity of them is more important in patients with hepatic encephalopathy history, who showed a higher frontal-subcortical dysfunction. Minimal hepatic encephalopathy may play a role in the severity of apraxia signs.

49 Alcoholic hepatitis decreases THE CAPACITY FOR urea synthesis

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Background and aims: Urea synthesis serves a key regulatory role in nitrogen (N) homeostasis. Its capacity decreases in patients with compromised liver function. In contrast, it increases in patients with inflammation. Alcoholic hepatitis (AH) involves both mechanisms, but it is unknown how their effects on urea synthesis are balanced. Our aim was to investigate how AH affects the capacity for urea synthesis.

Methods: We included twenty patients (m/f, 17/3; age 39-59 years) with a clinical diagnosis of AH. Eleven had severe AH: a Glasgow alcoholic hepatitis score (GASHS) ≥ 5. We measured blood a-amino-N concentrations (AAN) and urea-N synthesis rates (UNSR) before, during, and after a 4-h constant infusion of alanine (2mmol/kg/h). The capacity for urea synthesis was quantified by the
Functional Hepatic Nitrogen Clearance (FNHC), i.e. the slope of the linear dependence of UNSR on AAN. The FNHC was related to another metabolic liver function, the Galactose Elimination Capacity (GEC), and to clinical liver status assessed by the Model for End-Stage Liver Disease (MELD) and the Child-Pugh (CP) score.

**Results:** FNHC was markedly decreased to 7.2±4.9 l/h (mean±SD) in the patients (normal range 20-30 l/h) and most so in those with severe AH (4.9±3.5 l/h vs. 9.9±4.9 l/h, P<0.05). The GEC was less markedly reduced than the FNHC and they were dissociated. There was an inverse relation between the FNHC and the liver status scores (MELD r=0.49, P<0.05, C-P r=0.49, P<0.05).

**Conclusions:** Alcoholic hepatitis markedly decreases the capacity for urea synthesis and to a level previously only measured in acute liver failure. In AH, thus, the metabolic failure prevails so that the liver cannot appropriately deliver the metabolic up-regulation found in other stressful states including inflammation. This may contribute towards the frail prognosis of the patients.

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**50**

Do those with Minimal HE have significantly different diffusion-weighted tractography from healthy controls? Does a four-week trial of therapy alter this pattern?


**Objectives:** A) To identify microstructural white matter differences between patients with minimal hepatic encephalopathy (mHE) and healthy volunteers using diffusion-tensor imaging (DTI). B) To determine whether white matter microstructural changes occur in response to L-ornithine L-aspartate (LOLA) therapy in those with mHE.

**Methods:** 21 patients with biopsy-proven cirrhosis and MHE on psychiatry were treated with open-label LOLA and scanned at baseline, and after 4 weeks (12 female, mean age 47.1 years). 19 healthy volunteers were scanned at baseline (8 female, mean age 46.3 years). Diffusion-weighted MRI was obtained at 3T with 15 diffusion-encoding directions. Data were pre-processed in FMRIB software library (FSL) version 4. Tensor-based registration of images was performed using DTI-ToolKit. The ICBM-DTI81 white matter atlas was used to measure fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD) and the trace of diffusivity (TR). Statistical analysis was performed using the software XLSTAT 2014 (Addinsoft, Paris, France). Group differences in diffusion metrics were evaluated by analysis of covariance adjusted for age and sex.

**Results:** Lesion volume and anterior internal capsule had significantly different AD between patients and controls. No changes in DTI metrics were found after short-term LOLA treatment, relative to baseline measurements. This study suggests no short-term structural changes occur resolve when HE is treated. Further larger scale and longer-term treatment studies are required.

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**51**

Exchange of the liver - no change of life? A 1 Year follow up study AFTER liver transplantation

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**Introduction:** Employment after transplantation is considered an indicator of treatment quality. Socio-economic factors are esteemed crucial in this respect, but other factors such as hepatic encephalopathy (HE) prior to liver transplantation (OLT) might be important as well. In this prospective study employment status before OLT, occupation, education, history of HE, underlying liver disease, age and sex were analysed with regard to their impact upon the employment status after OLT.

**Methods:** 160 patients (age 49±12 years, n=96 (60%) male) underwent neurological and (if possible) neuropsychological examination and completed a questionnaire concerning occupation pre and 12 months post OLT.

**Results:** Eighty-nine of the patients were employed before OLT (55.6%). Of those 17 retired (19.1%) and 17 died (19.1%) after OLT. Patients who retired after OLT did not differ from those who continued working with regard to age, sex, underlying liver disease, history of HE, presence of neurological complications post OLT or professional category. Of 71 patients (44.4%) who were treated with OLT 3.4 (2.5–5.4) years post OLT. Research and Treatment Center Transplantation, ***Department of Gastroenterology, Hepatology and Endocrinology, ****Clinic for General, Abdominal and Transplant Surgery, Hannover Medical School, 30625 Hannover, Germany

**Background:** Hepatitis C virus (HCV) infection and its treatment with interferon and ribavirin (IFN-RBV) has been associated with significant levels of transaminase elevations and the development of severe hepatic injury. In this study, we evaluated the impact of IFN-RBV on liver function tests and the development of severe hepatic injury in chronic hepatitis C patients.

**Methods:** A total of 100 patients with chronic hepatitis C were randomly assigned to one of two groups: group A received IFN-RBV therapy and group B received placebo. Liver function tests were performed before and after 6 months of treatment and compared between the two groups.

**Results:** The results showed that IFN-RBV therapy resulted in significant improvement in liver function tests, including ALT, AST, and GGT compared to placebo. Moreover, the incidence of severe hepatic injury was significantly lower in the IFN-RBV group compared to the placebo group.

**Conclusion:** These findings suggest that IFN-RBV therapy may be beneficial in the treatment of chronic hepatitis C and should be considered as a potential treatment option for patients with severe hepatic injury.
Reduced expression of Glycine transporter 1 in cortical neurons of patients with acute liver failure

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Background: Astrocyte swelling and altered astrocyte-neuronal trafficking of amino acids are characteristics of central nervous system in acute liver failure (ALF). In addition alteration in N-methyl D-aspartate (NMDA) mediated neurotransmission has been involved in the pathogenesis of hepatic encephalopathy due to ALF. Glycine, a positive allosteric modulator required for regulation of NMDA receptor. One of the glycine transporters (GlyT)-1 is expressed in both astrocytes as well as neurons of the cerebral cortex in association with regions of high NMDA receptor expression. The present study investigated the expression of GlyT-1 in the frontal cortex of patients with ALF.

Methods: We analyzed the mRNA expression of GlyT-1 in the cerebral cortex obtained at autopsy from eight patients with ALF and from seven patients with no evidence of hepatic or neurological disorders by real-time PCR, and protein expression was assessed using immunoblotting and immunohistochemistry. The study groups were matched for gender (ALF, 4 men; control, 4 men) and post-mortem delay intervals [ALF: median 245 minutes (range 180-415); control median 240 minutes (135-870)]. All the ALF patients were in high grades of encephalopathy at the time of death and the same has been confirmed on histopathology.

Results: A significant decrease in GlyT-1 mRNA and protein levels was demonstrated in all ALF patients compared to controls. The loss of GlyT-1 protein in ALF samples was post-translational in nature. The immunostaining of GlyT-1 expression was decreased in ALF cortical brains compared to control brains and the GlyT-1 immunostaining was particularly expressed in neurons and not in astrocytes.

Conclusion: The above results suggest that decreased expression of the GlyT-1 transporter may cause an impairment of regulation of glycine concentration at synaptic level and contribute to an over-activation of the NMDA receptor in patients with ALF.
ABSTRACTS

**57** L-carnitine could improve minimal encephalopathy in cirrhotic patients
Author: Masaya Saito

**Background**
Liver cirrhotic patients with minimal hepatic encephalopathy (MHE) have a high risk of taking traffic accident, which is a social problem in the world. L-carnitine could improve mitochondrial function and subsequently activate urea cycle. We aimed to clarify whether L-carnitine could ameliorate MHE in cirrhotic patients.

**Methods**
We performed a prospective cohort study involving 51 cirrhotic out-patients at Kobe University Hospital. Neuropsychiatry test (NP test) and blood biochemical examinations were performed before and 3 months after L-carnitine administration. We examined 1) a morbidity of MHE in cirrhotic patients before L-carnitine administration, 2) an ameliorated rate of MHE at 3 months after the administration, and 3) an alteration of blood biochemical factors after the administration.

**Results**
In 51 cirrhotic patients, 18 patients (35%) had MHE before the administration. In 17 MHE patients, 9 patients (53%) showed the MHE amelioration at 3 months after the administration. In 32 cirrhotic patients, 13 patients (41%) showed an amelioration of serum ammonia (3 months after/ before the administration). The alteration of serum ammonia did not differ between MHE-ameliorated group and no-ameliorated group (P=0.441). The ratio of glutamine was significantly lower in MHE-ameliorated group than in no-ameliorated group (P=0.043).

**Conclusions**
L-carnitine could highly improve MHE in cirrhotic patients. The amelioration of serum glutamine level might be related to amelioration of MHE.

**58** Serum levels of l-carnitine associated with those of amino acids, fatty acid, and minerals in cirrhotic patients
Author: Hirotaka Hirano

**Introduction**
Recent studies showed that cirrhotic patients had impairment of l-carnitine utilization. Serum levels of l-carnitine were kept in normal irrespective of the extent of liver function. On the other hand, it has been unknown about serum factors associated with serum l-carnitine in cirrhotic patients. We aimed to clarify whether serum levels of l-carnitine was associated with those of amino acids, fatty acids, and minerals in cirrhotic patients.

**Patients and Methods**
We performed a prospective cohort study involving 50 cirrhotic out-patients at Kobe University Hospital. All of the patients did not take administration of l-carnitine, branched-chain amino acids and zinc supplements. Blood biochemical examinations were performed after overnight bed rest and fasting. We performed univariate analyses between serum levels of l-carnitine and those of amino acids, fatty acids, and minerals. We also performed multiple regression analyses using the significant factors.

**Results**
In multiple regression analyses, serum level of acylcarnitine was positively associated with that of free fatty acid (FFA) in cirrhotic patients (p=0.019). Serum level of total carnitine was positively associated with that of cystine (p=0.000), and was negatively associated with that of sodium (p=0.013).

**Discussion**
L-carnitine is important for the oxidation of fatty acids. In cirrhotic patients, the impaired l-carnitine utilization would induce the decreased oxidation of fatty acid, following higher serum level of FFA. In addition, l-carnitine promotes the production of the anti-oxidant glutathione. Cystein also has neuroprotective effects by antioxidant activity. Cystein easily transforms into cystine by oxidation in protein extraction process. They can cross blood brain barrier (BBB). In cirrhotic patients, the impaired l-carnitine utilization might induce higher serum level of cystine because of the impairment of BBB transition.

L-carnitine could highly improve MHE in cirrhotic patients. The amelioration of serum glutamine level might be related to amelioration of MHE.

**56** Serotonin and blood brain barrier within circumventricular organ and hepatic encephalopathy
Samir ABOUCHA
Hassan First University, Faculty of Khouribga, Khouribga, Morocco samir.aboucha@uhp.ac.ma

Blood brain barrier includes several components such as tight junctions, transporter uptake through endothelial cells, as well as the functional state of particular structures known as circumventricular organs (CVO). Changes of neuroendocrine systems is related to hepatic encephalopathy (HE) including the serotonergic (5-HT) system, which plays a role in neurotransmission and brain vasomodulation. In HE, there is evidence for an inhibition of the firing rate of 5-HT neurons, and several attempts to overcome this situation uses activation of the firing of 5-HT neurons using 5-HT antagonists or drugs that reduce 5-HT concentrations at the synaptic cleft. For example, administration of the nonselective 5- HT antagonist methysergide to rats with thioacetamide-induced ALF ameliorates motor activity in these animals, while 5-HT3 receptor antagonist ondansetron induces improvement of locomotor performances in bile duct ligated (BDL) rats. Moreover, administration of agonists of the 5-HT1A subtype, a presynaptic auto- receptor-which when activated results in decreased 5-HT release in the brain and subsequent reductions of 5-HT at the synaptic cleft - attenuates motor behaviour in BDL rats. It is well established that 5-HT reduces 5-HT release in the brain and subsequent reductions of 5-HT at the synaptic cleft. We hypothesize 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 material aggregation in cerebrospinal fluid and brain parenchyma through altered BBB.

Keywords: Subcommissural organ, Serotonin, Hepatic encephalopathy, Immunohistochemistry, Rat.

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**Table. Comparison between the patients included in the present study and the historical controls (Riggio o. et al. J Hepatol. 2010).**

<table>
<thead>
<tr>
<th>Albumin treatment (n=23)</th>
<th>Historical Controls (n=45)</th>
<th>P</th>
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<tr>
<td>n (%)</td>
<td>n (%)</td>
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**57** L-carnitine could improve minimal encephalopathy in cirrhotic patients
Author: Masaya Saito

**Background**
Liver cirrhotic patients with minimal hepatic encephalopathy (MHE) have a high risk of taking traffic accident, which is a social problem in the world. L-carnitine could improve mitochondrial function and subsequently activate urea cycle. We aimed to clarify whether L-carnitine could ameliorate MHE in cirrhotic patients.

**Methods**
We performed a prospective cohort study involving 51 cirrhotic out-patients at Kobe University Hospital. Neuropsychiatry test (NP test) and blood biochemical examinations were performed before and 3 months after L-carnitine administration. We examined 1) a morbidity of MHE in cirrhotic patients before L-carnitine administration, 2) an ameliorated rate of MHE at 3 months after the administration, and 3) an alteration of blood biochemical factors after the administration.

**Results**
In 51 cirrhotic patients, 18 patients (35%) had MHE before the administration. In 17 MHE patients, 9 patients (53%) showed the MHE amelioration at 3 months after the administration. In 32 cirrhotic patients, 13 patients (41%) showed an amelioration of serum ammonia (3 months after/ before the administration). The alteration of serum ammonia did not differ between MHE-ameliorated group and no-ameliorated group (P=0.441). The ratio of glutamine was significantly lower in MHE-ameliorated group than in no-ameliorated group (P=0.043).

**Conclusions**
L-carnitine could highly improve MHE in cirrhotic patients. The amelioration of serum glutamine level might be related to amelioration of MHE.

**58** Serum levels of l-carnitine associated with those of amino acids, fatty acid, and minerals in cirrhotic patients
Author: Hirotaka Hirano

**Introduction**
Recent studies showed that cirrhotic patients had impairment of l-carnitine utilization. Serum levels of l-carnitine were kept in normal irrespective of the extent of liver function. On the other hand, it has been unknown about serum factors associated with serum l-carnitine in cirrhotic patients. We aimed to clarify whether serum levels of l-carnitine was associated with those of amino acids, fatty acids, and minerals in cirrhotic patients.

**Patients and Methods**
We performed a prospective cohort study involving 50 cirrhotic out-patients at Kobe University Hospital. All of the patients did not take administration of l-carnitine, branched-chain amino acids and zinc supplements. Blood biochemical examinations were performed after overnight bed rest and fasting. We performed univariate analyses between serum levels of l-carnitine and those of amino acids, fatty acids, and minerals. We also performed multiple regression analyses using the significant factors.

**Results**
In multiple regression analyses, serum level of acylcarnitine was positively associated with that of free fatty acid (FFA) in cirrhotic patients (p=0.019). Serum level of total carnitine was positively associated with that of cystine (p=0.000), and was negatively associated with that of sodium (p=0.013).

**Discussion**
L-carnitine is important for the oxidation of fatty acids. In cirrhotic patients, the impaired l-carnitine utilization would induce the decreased oxidation of fatty acid, following higher serum level of FFA. In addition, l-carnitine promotes the production of the anti-oxidant glutathione. Cystein also has neuroprotective effects by antioxidant activity. Cystein easily transforms into cystine by oxidation in protein extraction process. They can cross blood brain barrier (BBB). In cirrhotic patients, the impaired l-carnitine utilization might induce higher serum level of cystine because of the impairment of BBB transition.

L-carnitine could highly improve MHE in cirrhotic patients. The amelioration of serum glutamine level might be related to amelioration of MHE.

**56** Serotonin and blood brain barrier within circumventricular organ and hepatic encephalopathy
Samir ABOUCHA
Hassan First University, Faculty of Khouribga, Khouribga, Morocco samir.aboucha@uhp.ac.ma

Blood brain barrier includes several components such as tight junctions, transporter uptake through endothelial cells, as well as the functional state of particular structures known as circumventricular organs (CVO). Changes of neuroendocrine systems is related to hepatic encephalopathy (HE) including the serotonergic (5-HT) system, which plays a role in neurotransmission and brain vasomodulation. In HE, there is evidence for an inhibition of the firing rate of 5-HT neurons, and several attempts to overcome this situation uses activation of the firing of 5-HT neurons using 5-HT antagonists or drugs that reduce 5-HT concentrations at the synaptic cleft. For example, administration of the nonselective 5- HT antagonist methysergide to rats with thioacetamide-induced ALF ameliorates motor activity in these animals, while 5-HT3 receptor antagonist ondansetron induces improvement of locomotor performances in bile duct ligated (BDL) rats. Moreover, administration of agonists of the 5-HT1A subtype, a presynaptic auto- receptor-which when activated results in decreased 5-HT release in the brain and subsequent reductions of 5-HT at the synaptic cleft - attenuates motor behaviour in BDL rats. It is well established that 5-HT reduces 5-HT release in the brain and subsequent reductions of 5-HT at the synaptic cleft. We hypothesize 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 material aggregation in cerebrospinal fluid and brain parenchyma through altered BBB.

Keywords: Subcommissural organ, Serotonin, Hepatic encephalopathy, Immunohistochemistry, Rat.
Background and aims: Interferon(IFN) therapy is important treatment of chronic hepatitis C(CH-C) patients. However, adverse effects of this therapy that depression or neuropsychiatric symptoms make it difficult to be completed. Methods: The aim of study is to examine neuropsychiatric symptoms with IFN therapy and its correlation of effects on cerebral glucose metabolism(CMRglu) in CH-C patients. 13 CH-C patients undergoing IFN therapy (Peg Interferons+Ribavirin or Interferons monotherapy) were evaluated neuropsychiatric symptoms by neuropsychiatric test such as Digit symbol test(DST), Block design test(BDT) and Self-rating Depression Scale(SDS). We assessed CMRglu using [18F] deoxyglucose positron emission tomography (FDG-PET). On basic science, we examined human astrocytes were exposed to IFNs, and their proliferation and the glucose consumption. Results: Compare to before and 8ths weeks of IFN therapy, SDS of all patients were worsened. CMRglu of 8 patients were 1-24% decreased in whole of the brain. CMRglu of 5 patients were 2-37% increased in whole of the brain. There were no trend of result that DST and BDT before and 8ths weeks of therapy. We examined 7 patients completed the therapy. Compare to before and after the therapy, SDS of all patients after the therapy were recovered same as before. CMRglu of 6 patients were 11-73% increased in whole of the brain from before the therapy. CMRglu of 1 patient were recovered in whole of the brain same as before. On basic science, treatment with IFNα2a and IFNα2b significantly decreased the proliferation of cultured human astrocytes by 23% (p=0.007) and 13% (p=0.02), and decreased the glucose consumption in cultured human astrocytes. Conclusions: These results suggest IFN therapy affect on cerebral glucose metabolism and depression that are due to effects of IFNs on proliferation and glucose utilization in human astrocytes.

60 Diabetic and hepatic encephalopathy: similarities, synergisms and differences Freimut Schliess, Director Scientific Affairs, Profil Neuss, Germany

According the International Diabetes Federations' most recent estimate 382 million people (8.2% of the adults) have diabetes mellitus (DM), and the number is expected to increase to 592 million people in less than 25 years. By widening the traditional glucocentric view subclinical inflammation and reactive oxygen and nitrogen species are increasingly recognised drivers of insulin resistance, beta cell dysfunction and micro-macrovacular complications. Diabetes has been associated with a 44% acceleration of mental decline and a 65% increase in the risk of incident Alzheimer disease (AD). As brain insulin resistance is an early and common feature AD has been hypothesised to be a type T1DM. Diabetic encephalopathy (DE) is an increasingly explored complication of both, T1 and T2DM, which critically affects physical and mental integrity particularly in the elderly. Cerebral inflammatory and oxidative stress triggered by recurrent hypoglycemia, C-peptide depletion (T1DM) and pro-diabetic risk factors (T2DM) are key in mediating biochemical and structural brain changes affecting both neurons and astrocytes. Diabetes aggravates hepatic encephalopathy (HE) and liver cirrhosis predisposes to diabetes. Against this background the overview will provide a comprehensive summary on the interaction between DM and liver disease in the evolution of cognitive and mental impairment. Special attention will be paid to molecular pathogenesis and its potential interference by current diabetes therapies.

61 Spectral electroencephalogram analysis in liver cirrhosis with minimal hepatic encephalopathy before and after lactulose therapy Jatinpal Singh, Barjesh Chander Sharma, Vinod Puri*, Siddharth Srivastava Department of Gastroenterology and Neurology*, G B Pant Hospital, New Delhi, India

Background/Aims: Minimal Hepatic Encephalopathy (MHE) represents the mildest form of Hepatic Encephalopathy (HE) with no clinically overt overt HE but alterations in specific laboratory test results that include psychometric test results and Electroencephalograph (EEG). Spectral EEG analysis (sEEG) may further improve the recognition of MHE by decreasing interoperator variability and providing quantitative parameters of brain dysfunction.Variou studies has provided evidence of reduced lactate and general well being in cirrhotic patients. In this study we have compared sEEG in patients of cirrhosis with and without MHE and the effect of lactulose therapy on sEEG in patients with MHE.

Methods: 50 patients of cirrhosis (25 with MHE and 25 without MHE) were enrolled. Assessment of MHE was done by psychometric tests. sEEG was performed at baseline in all cirrhotics. After a visual inspection to exclude artefacts, the EEG tracing assessed by spectral analysis of 2 second epochs over a period of 60s. Spectral analysis carried out on the derivation in the frequency range 1–25.5 Hz. The spectral variables considered were the mean dominant frequency (MDF), i.e., the mean frequency weighted by the power of each frequency band and the relative power in the beta (13.5–25.5 Hz), alpha (8.5–13 Hz), theta (4–8 Hz) and delta (1–3.5 Hz) bands. Patients with MHE were given 3 months of lactulose therapy and psychometric tests and sEEG was repeated in them.Statistical tests used as appropriate.

Results: MDF values found to be lower in cirrhotics with MHE (7.9±1.1Hz) as compared to cirrhotics without MHE (8.4±1.2Hz,p=0.06). Theta % was higher in patients with MHE than in patients without MHE (31.7±12% vs 24±6.7%, p=0.001). However no significant difference was found in alpha,beta and delta bands. After treatment of 3 months with lactulose significant improvement is seen in MDF and theta % in patients with MHE (p≤0.05).

Conclusions: Spectral EEG is a useful measure for diagnosing minimal hepatic encephalopathy and monitoring improvement or worsening of minimal hepatic encephalopathy with treatment in patients with cirrhosis.


BACKGROUND AND AIMS: In cirrhotic patients an alteration in gut microbiota has been characterized by an overgrowth of Enterobacteriaceae (e.g. E.coli) and a decrease in autchothonous familie such as the Clostridiacae (i.e.Faecalibacterium prausnitzii). This dysbiosis has been recently associated with systemic complications, such as hepatic encephalopathy.

AIMS: We aimed to correlate the microbiome features with cirrhosis and the pro-inflammatory condition in these patients.

METHODS: Patients were excluded if their histories included antibiotic treatment within the past 4 weeks or if they were taking lactulose. Temporal Temperature Gradient Gel Electrophoresis (TTGE) was performed on stool specimens of cirrhotic patients and age-matched controls. The F.prausnitzii/E.coli ratio, assessed by quantitative PCR (qPCR), was used to define gut dysbiosis. TTGE patterns were compared by means of χ2, while qPCR results were compared through Mann-Whitney U test. The importance of variables in defining SiRS status was computed with a custom R module, and tested with Pearson's correlation coefficient. The diagnosis of SiRS was made according to guidelines.

RESULTS: Twenty-seven cirrhotic patients (17 Child B/C) and nine controls were included. Cirrhotic faeces, compared to controls, showed different TTGE profiles (P=0.0022) (figure 1). As shown in figure 2, at a-PCR cirrhotic faeces showed higher levels of E.coli (p<0.05), while controls had higher levels of F.prausnitzii (p>0.05). Cirrhotic patients had a significant reduction of the F.prausnitzii/E.coli ratio (p=0.0004). We also found a relative higher concentration of Enterobacteriaceae and a lower concentration of Clostridiaceae group XIV in cirrhotic patients Vs. controls. Within the cirrhotic group, E. coli levels showed a positive correlation with C-reactive protein (r2=0.54; P=0.05), synchrocytes of E.coli (r2=0.48; P=0.05) and cardiac frequency (r2=0.58; P=0.05). Ten cirrhotics were diagnosed to have a SiRS. Logistic regression showed a trend to significance between SiRS and dysbiosis (C1: 0.8-1.7; p=0.06).

CONCLUSIONS: Cirrhosis is associated with significant alterations in stool microbiome. Specific bacterial imbalance, expressed by the F.prausnitzii/E.coli ratio, is associated with signs of the pro-inflammatory condition present in cirrhotic patients.

Figure 1.

Figure 2.

Figure 3.
ABSTRACTS

63 Clinical significance of minimal hepatic encephalopathy
Galvin Z, Dillon A, Lowry D, Russell J, Stewart S.
Mater Misericordiae University Hospital

Background
Minimal hepatic encephalopathy (mHE) is the primary cause of cognitive deficits in patients with cirrhosis. It has been reported that these patients are more likely to develop overt hepatic encephalopathy and are less likely to survive for the same length of time as their unimpaired counterparts.

Aim
To investigate if mHE is associated with poorer outcomes in a cohort of compensated cirrhotic patients and also to determine which psychometric test correlates best with patient outcome.

Methods
Consecutive compensated cirrhotic patients attending the outpatient department over a two year period were recruited for the study. Psychometric testing, including the psychometric hepatic encephalopathy score (PHES), the repeatable battery for assessment of neuropsychological status (RBANS) and the critical flicker fusion (CFF) test, were performed at each visit. For PHES and RBANS, mHE was diagnosed in clinically unimpaired patients who scored <= two standard deviations (sd) below the mean for a normative population. For CFF, mHE was diagnosed in clinically unimpaired patients who had a CFF of <8Hz. The study endpoints were the development of ascites, hepatic encephalopathy, bleeding varices or death.

Results
Of 124 patients, 11 patients decompensated/had a liver-related death during the study (median follow up 15.4months). On univariate analysis mHE, as diagnosed by the PHES and not as diagnosed by RBANS or CFF, was significantly associated with death/decompensation (p=0.05) (Kaplan Meier analysis Log rank 4.23; p=0.04). The PHES total score was then dichotomised according to a cut-off point identified by a ROC curve. A cut-off threshold of -2.72 sd below the mean was chosen as the score that gave the highest sum of sensitivity and specificity to predict decompensation or death. A Kaplan Meier analysis, using this threshold to divide the cohort into two groups, was significantly associated with death or decompensation (Log rank 16.18; p=0.00006).

Conclusion
Compensated cirrhotic patients with mHE, diagnosed by PHES, are more likely to decompensate and less likely to survive than compensated cirrhotic patients without mHE. Using a cut-off threshold that is lower than that used to diagnose mHE appears to offer an advantage in terms of predicting likelihood of decompensation or death.

64 Pathophysiological basis of Hepatic Encephalopathy (HE) in patients with Acute-on-chronic liver failure (ACLF): A prospective, longitudinal study determining the role of ammonia, inflammation and cerebral oxygenation
R Sawhney, P Holland-Fischer, R Mookerjee, M Roselli, B Agarwal, R Jalan

Background: HE is a feature of acute liver failure and is associated with high mortality in ACLF. Although ammonia, inflammation, cerebral perfusion and oxygenation are associated with HE in ALF, their roles in ACLF patients are unknown. The aim of this prospective, longitudinal observational study was to determine the role of this pathophysiological variables in ACLF patients with or without HE.

Methods: 101 patients (MF: 69/32; mean age: 54; Alcohol: 78%) with ACLF admitted to ICU were studied. Severity of ACLF was classified using the CLIF-SOFA score and severity of HE using West-Haven criteria. All patients were managed according to a pre-defined protocol and organ support provided as required. Arterial ammonia, jugular venous oxygen saturation (JVO2), white cell count (WCC) and CRP were measured at time of enrollment, days, 1, 3 and 7 or, until death/discharge.

Results: 51 patients died (50.5%). Mortality was higher in ACLF patients with HE (ACLF-HE) irrespective of severity of ACLF (ACLF-HE: 35/53 (66%); ACLF-no HE: 16/48 (33%); p=0.001). Mortality was greater in patients with greater severity of HE (Grade 0/1: 16/48 (33%); Grade 2: 19/32 (60%) Grade 3: 10/18 (56%); p=0.002). INR, creatinine, WCC, low platelets at baseline, and ACLF severity were independent predictors of death in the whole and ACLF-HE cohorts. Baseline ammonia levels were higher in HE patients (90 vs 73 µmol/L; p=0.004) but did not predict mortality. A decrease in ammonia was associated with better survival (p<0.001). Abnormal baseline JVO2 (deviation by more than 5% from an optimal 75%) was associated with both presence and severity of HE (ACLF-no HE: 22%; ACLF-HE Grade 2: 47%; ACLF-HE Grade 3-4: 62%; p=0.005). Worsening JVO2 (low or high) was independently associated with mortality (improved JVO2: 21% mortality; worsened 79%; p=0.001). WCC did not differ between non-HE and HE groups at baseline (p=0.95) but WCC was higher in those that died (p=0.007). A further increase was independently predictive of death (p<0.001). There was a strong interaction between ammonia and JVO2 in regards to predicting severity of HE and mortality.

Conclusions: This study describes potential mechanisms of HE in ACLF indicating that ammonia and abnormal cerebral oxygenation are important. These findings suggest that ammonia, JVO2 and WCC are important prognostic biomarkers and therapeutic targets. Whether altered JVO2 is independent of ammonia in the pathogenesis of HE in ACLF requires future study.

O1 Hyperpolarized pyruvate allows early detection of lactate in real-time metabolism of ALF rats.

Laia Chavarri1,2, Jordi Romero-Giménez1, Eva Monteagudo1, Silvia Lope-Piedra1,4, Juan Cordoba1,2,3
1Liver Unit, Hospital Vall Hebron, Barcelona, 2Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERHED), 3Departament Medicina. Universitat Autònoma de Barcelona, Bellaterra, 4Servei de RMN, Universitat Autonoma de Barcelona, Bellaterra, Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), SPAIN

Background and Aim:
Intracranial hypertension is a severe complication of acute liver failure (ALF) secondary to brain oedema. The pathogenesis of cerebral oedema in ALF is not clear but energy metabolism alterations are involved where a genesis of lactate seems to have an important role. The aim of the study was to follow the dynamic synthesis of brain metabolites using hyperpolarized pyruvate in a model of ALF.

Methods:
Animal Model: Acute liver failure (ALF) was induced in Sprague-Dawley male rats (250-300g) by porto-cava anastomosis (PCA) and hepatic artery ligation. This model is characterized by highly predictable course: the precoma stage is defined as loss of the righting reflex and coma stage as loss of corneal reflex. Animals were anesthetized with isoflurane and body temperature was kept constant at 37±0.5°C. The metabolism was assessed by 13C-hyperpolarized pyruvate. This study was performed on PCA and ALF rats (n=8) repeatedly at 6 and 12 hours after sham or HAL surgery, respectively. The composition of the hyperpolarized pyruvate was 6 and 12 hours after sham or HAL surgery, respectively.

Results:
Intracranial hypertension is a severe complication of acute liver failure (ALF) secondary to brain oedema. The pathogenesis of cerebral oedema in ALF is not clear but energy metabolism alterations are involved where a genesis of lactate seems to have an important role. The aim of the study was to follow the dynamic synthesis of brain metabolites using hyperpolarized pyruvate in a model of ALF.

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O2

Bile-ligated rats are susceptible to hypotension-induced neuronal cell loss: Implications for persisting neurological complications following liver transplantation

Marc-André Clément, Cristina Bosoi, Mélanie Tremblay, Chantal Béméur and Christopher Rose

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Background: Hepatic encephalopathy (HE) is a major neuropsychiatric complication caused by liver disease characterized by cognitive and motor dysfunction. The only curative treatment to date remains liver transplantation (LT). Historically, HE has always been considered to be a reversible metabolic disorder and has therefore been expected to completely resolve following LT. However, even following the implantation of a new liver, persisting neurological complications remain a common problem affecting as many as 47% (8-47%) of liver transplant recipients. LT is a major surgical procedure accompanied by intraoperative stress and confounding factors, including blood loss (hypovolaemia) and hypotension. We hypothesize, in the setting of MHE, that the compromised brain becomes predisposed to what would normally be an innocuous hypotensive insult, resulting in cell injury and death.

Methods: Using 6-week bile-duct ligated rats and respective controls, blood is withdrawn from the femoral artery (inducing hypovolaemia) until an arterial pressure of 30 mmHg (hypotension) and maintained for 150 minutes. Upon sacrifice, brains are perfused and extracted for western blotting and immunohistochemistry.

Results: Both BDL rats and SHAM-operated controls without hypotension do not display any neuronal loss. However, BDL rats following hypotension demonstrated a significant decrease in neuronal cell count in the frontal cortex using NeuN+DAPI and Cresyl Violet compared to hypotensive SHAM-operated controls. In addition, neuronal loss was associated with an increased in cellular stress protein, hsps2, hsps70 and caspase-3, suggesting apoptotic cell death.

Discussion: These findings suggest that patients with HE are more susceptible to hypotension-induced neuronal cell loss and this may explain why transplanted patients are experiencing persisting neurological complications. Aside from cirrhotic patients having a stroke, these results also suggest a patient with HE (even MHE) with a "frail brain", fare worse during transplantation leading to poor neurological outcome. This implies MHE should not be ignored and therefore treated pre-LT.

References

O3

Effects of chronic hepatic encephalopathy on brain energy metabolism, studied by in vivo 13C MRS in rats

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5 Service of Biomedicine, University Hospital of Lausanne, Switzerland.

Introduction

Increased brain ammonia generated by chronic liver disease (CLD) is detoxified mainly in astrocytes through glutamine synthetase activity, leading to increased CNS glutamate, astrocyte swelling and consequently making astrocytes the primary target of ammonia neurotoxicity. In this context, our aim was to identify if, at latest stages, increased glutamine impacts brain energy metabolism by applying in vivo dynamic 13C-MRS together with administration of [1,6-13C2]-glucose and appropriate metabolic modelling [1].

Methods

Localized 13C spectra were measured in the brain of control (n=5) and CLD (n=7) Wistar rats. The CLD model was created by bile-duct-ligating (BDL) the animals 8 weeks before 13C acquisition. [1,6-13C2]-glucose was infused to maintain 70% enrichment in blood glucose for 5h. 13C labeling of glutamate and glutamine was followed over time to determine the kinetics of glial and neuronal TCA cycles and glutamate/glutamine cycle activity. All data were acquired on a 9.4T system (Varian/Magnex Scientific) using a home-built 10mm (13C)/13mm (1H quad) surface coil as RF transceiver and the semi-adaptive DEPT polarization transfer sequence (TR=2.5s, interpulse delay 3.8ms (JCH=130Hz), 45° for last 1H pulse to simultaneously measure signals from CH, CH2, CH3 groups) [2]. 1H spectra were acquired in the same VOI before the glucose injection to measure glutamate (Glu) and glutamine (Gln) pool sizes.

Results and Discussion

No major flux changes were observed between the two groups, except for the glial dilution at the level of Acetyl-CoA, which was significantly stronger in the BDL group. This suggests a shift of glial metabolism to alternative energy substrates, such as acetate or fatty acids. The glutamate/glutamine cycle was preserved.

Our 13C-MRS study reveals that although significant changes in amino acids and osmolytes concentrations were measured at this stage of the disease in previous studies, no significant effect on oxidative metabolism and apparent neurotransmission was observed, suggesting that mild brain edema in CLD-induced hepatic encephalopathy is primarily caused by other effects of hyperammonemia, such as osmotic imbalance and resulting cell swelling.

References

O4

Hyperammonaemia and systemic inflammation is associated with intracerebral lactate accumulation and preserved respiratory capacity in brain tissue of rats.

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Background and aim: In acute liver failure (ALF) cerebral oedema and high intracranial pressure (ICP) are potentially deadly complications. In vitro studies of astrocyte cultures have shown mitochondrial dysfunction under hyperammonaemic conditions and in rat brain of in vivo liver failure models de novo lactate production has been observed. These findings support the hypothesis of a compromised brain metabolism during ALF. Yet, normal lactate levels are found in cerebral microdialysate of ALF patients and the oxygen to glucose ratio of cerebral metabolic rates remains normal. We therefore wanted to investigate the relationship between the extracellular and total lactate levels in brain cortex of a rat model with hyperammonaemia and systemic inflammation. Furthermore we assessed the mitochondrial function in brain tissue with high-resolution respirometry.

Methods: Sedated and mechanically ventilated male Wistar rats were given either: ammonia (NH3)+lipopolysaccharide (LPS): NH3+saline; saline+LPS; or saline+saline. Ammonia/saline was infused for 120 minutes while extracellular brain lactate was measured with enzymatic biosensors (Sarissa Biomedical). After the animals were sacrificed the total lactate concentration in cerebral cortex was measured with respirometry (Oroboros Instruments), concretely the function of complex I (state 2+3), complex I+II and after uncoupling. Results: Injection of NH3 and LPS resulted in hyperammonaemia (1550±147µM vs. control 485±5µM, p<0.01). This was associated with a significantly elevated intracranial pressure (6.8±2.1mmHg vs. control 2.0±0.4mmHg, p<0.05). The total cerebral lactate level increased (20.0±3.4 mM vs. control 12.3±1.7 mM, p<0.05). There was no increase in the extracellular lactate, but a tendency towards lower levels in rats given ammonia and LPS (6.3±2.2±2.3µM vs. control 8.3±7.9µM, NS) (Figure). We did not find a significant reduction in the respiratory capacity of brain cortex in any of the studied respiratory states. Conclusion: Hyperammonaemia and systemic inflammation in rats was associated with increased total brain lactate and elevated ICP. We observed that the extracellular lactate levels remained normal and thereby indirectly demonstrated that the lactate accumulation was intracellular. Apparently, the pathophysiology did not involve reduced respiratory capacity indicating that the mitochondrial function was preserved.
ABSTRACTS

O5 Increase in plasma levels of brain specific microRNA-124-1 associates with falling cerebral perfusion pressure in an acetaminophen-induced porcine model of acute liver failure

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Aim: To investigate the potential use of miR124-1 as a plasma biomarker of brain injury in ALF.

Methods: A pig model of acetaminophen (APAP)-induced ALF (Liver Int. 2013; 33: 544) was used for this study with six APAP and three control pigs. After onset of APAP dosing, peak serum APAP levels (>350mg/l) occurred at 12h and ALF (International Normalised Ratio > 3) at 19±2h. Intracranial hypertension and death occurred 13±3h after ALF. Plasma and tissue samples were collected every 4h and at post mortem respectively. RNA was extracted and small RNA-enriched fractions were separated from larger RNAs using miRNeasy kit and assessed qualitatively and quantitatively on small RNA chips. miR124-1 and the endogenous control miR26a, were tested for amplification efficiency and quantified by RT-qPCR.

Results: miR124-1 was highly expressed in porcine brain tissue. Plasma levels of miR124-1 increased significantly (P < 0.001) immediately prior to death (13±3h) compared to controls. In addition, increasing plasma miR124-1 levels associated significantly with falling cerebral perfusion pressure (CPP; P = 0.010) compared to controls. This effect was correlated with a reduction in MTS activity and worsening of neurological outcomes.

Conclusions: This study shows the temporal association between rising plasma ammonia, increasing ICP, falling CPP and increasing plasma levels of brain specific miR124-1 in a porcine model of APAP-induced ALF. Further investigation with a suitable human population is warranted to establish the potential of brain specific miR124-1 in a porcine model of APAP-induced ALF.

Background/Aim

Hyperammonemic Effect in Primary Human Hepatic Stellate Cells

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Ammonia is a nitrogen waste product of protein metabolism. An increased level of ammonia induces numerous neurological disorders. Ammonia is hydrophilic and easily transported in the plasma and recent observations suggest that a reduction in its level can ameliorate the severity of portal hypertension. The mechanism of ammonia induced cytotoxicity in primary human stellate cells (NH4Cl) is still not fully understood.

Materials and methods

Primary NH4Cl isolated from human liver and ammonia-induced cell death was assessed by fluorescence activation of the VX2 cells, a human HSC cell line. Cells were cultured under basic serum-rich conditions for 24 hours, followed by serum deprivation for another 24 hours. Moreover, cell culture medium was devoid of glutamine and this to avoid interference with glutamine normally present in the basic medium. Cells were then exposed to different ammonium chloride concentrations (from 0.1 mM to 10 mM) as a single or repeated treatment for different time points up to 72 hours. Endpoints measured included proliferation by BrdU incorporation and metabolic activity by employing tetrazolium MTS. Possible changes in morphology due to ammonia were evaluated by time course microscopic observations.

Results

No effect of ammonia was detectable in the first 24 hours of treatment in primary NH4Cl and VX2 cells. However, visible changes in cell morphology, such as cell swelling and induction of a more fibroblast-like phenotype, were observed after 48 hours in a dose dependent manner (in a single or repeated ammonia treatment). This effect was correlated with a reduction in MTS metabolism and proliferation rate when compared to serum-and glutamine deprived control cells. No cell detachment was detectable in all concentrations/ time points under investigation. Protein analysis of NH4Cl specific signalling pathways and collagen gel contraction assays are ongoing.

Conclusion

These data suggest that ammonia affects primary NH4Cl and LX2 cells by interfering with metabolic activity and cell proliferation, but not cell viability. Thus ammonia and hyperammonemia might influence hHSC cell biology and therefore be important in the pathogenesis of portal hypertension.

O7 Dysregulation of the monocyte chemoattractant protein 1/fractalkine balance in hepatic encephalopathy following acute liver failure: role of bile acid signaling

Matthew McMillin, Gabriel F rampton, Cheryl Galindo, Sharon DeMorrow

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Introduction: Neuroinflammation is an integral component of hepatic encephalopathy (HE). The chemokine monocyte chemoattractant protein 1 (MCP-1), via binding chemokine receptors 2 (CCR2) and 4 (CCR4), regulates microglial activation and has been implicated in HE due to both chronic and acute liver injury. Conversely, the chemokine fractalkine (FKN) is highly expressed in the brain and serves as a suppressor of microglia activation. Bile acids have previously been shown to regulate inflammatory processes in the liver. We have shown that bile acids access the brain and contribute to the pathogenesis of HE. The exact mechanisms by which this is perpetuated by an inflammatory response, and ultimately results in multi-organ failure following acute liver injury are less clear.

Methods: HE was induced by injecting male C57Bl6 mice with ammonium chloride (AOM) (100µg/g ip) in the presence of CCR2 and CCR4 antagonists or omission of bile acid signaling in a murine model of HE.

Results: MCP-1 was upregulated and FKN was downregulated in frontal cortex neurons rapidly following AOM injection. Pretreatment of AOM-injected mice with CCR2 and CCR4 antagonists delayed neurological decline and microglia activation implicating MCP-1 signaling in HE. Treatment of primary neurons with CA and TCA increased MCP-1 expression and decreased FKN expression. Cholesteromyel eating reduced serum and brain bile acid levels and delayed neurological decline without altered liver damage observed after AOM injection. Furthermore, cholesteromyel eating prevented the AOM-induced increase in MCP-1 and decrease in FKN, and suppressed microglia activation.

Conclusion: Our data demonstrates that bile acids facilitate an imbalance between MCP-1 and FKN, which leads to a proinflammatory environment. Targeting bile acid, FKN or MCP-1 signaling may prove to be viable options for the management of HE.

O8 Effects of HE-relevant factors on the expression of the multidrug resistance protein 4

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Introduction: The multidrug resistant protein 4 (Mrp4/ABCC4) is a member of the ATP-binding cassette (ABC) transporter family. In humans, Mrp4 is expressed in different organs such as kidney, liver, gut and brain. In brain, Mrp4 is mainly expressed by endothelial cells constituting the blood brain barrier, but also by neurons and astrocytes. Presently, the function of Mrp4 in brain is not fully understood. However, Mrp4 substrates such as neurotransmitters, prostanoids and glutathione are also involved in the pathogenesis of hepatic encephalopathy. Therefore, we investigated effects of HE-relevant factors such as ammonia, benzodiazepines, inflammatory cytokines and hyperoosmolality on Mrp4 expression in primary cultured rat astrocytes.

Methods: In rat brain, Mrp4 is expressed in astrocytes, neurons and endothelial cells at the blood brain barrier as shown by immunofluorescence analysis. Treatment of cultured rat astrocytes with NH4Cl (5mMol, 72h) upregulated Mrp4 mRNA and protein expression levels by about 3-fold of untreated controls. Upregulation of Mrp4 mRNA and protein levels in NH4Cl (5mMol, 72h) treated astrocytes was sensitive towards the glutamine synthetase inhibitor methionine-sulfoximine (3mMol). In contrast, Mrp4 expression decreased in primary human Hepatic Stellate Cells; hyperammonemia.

O6 Dysregulation of the monocyte chemoattractant protein 1/fractalkine balance in hepatic encephalopathy following acute liver failure: role of bile acid signaling

Matthew McMillin, Gabriel F rampton, Cheryl Galindo, Sharon DeMorrow
remaining unchanged in astrocytes treated with CH$_3$NH$_2$Cl (5mmol/l, 72h), hypoosmolality (205 mosmol/l, 72h), the benzoazadipine diazepam or the pro-inflammatory cytokine TNF-a (10ng/ml, 72h).

Treating astrocytes with NH$_4$Cl (5mmol/l, 72h) reduced molecular mass of Mrp4 similar to treatment with peptide-N-glycosidase (PNGase), suggestive for inhibition of Mrp4 N-glycosylation by NH$_4$Cl.

As shown by Western-blot and gene array analysis, Mrp4 protein and mRNA levels were also elevated in post mortem brain biopsies of patients with liver cirrhosis and HE, but not in patients with cirrhosis without HE or controls.

Discussion: Mrp4/MRP4 becomes upregulated in ammonia-treated astrocytes and in human brain in hepatic encephalopathy. Increased Mrp4 protein expression in brain of patients with liver cirrhosis and HE suggests an important role for this transporter in the pathogenesis of HE.

O9
L-Ontinine Phenylacetate reduces ammonia in pigs with acute liver failure through phenylacetylglutamine formation: a novel ammonia-lowering pathway
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Background: Glycine is an important ammoniagenic amino acid, which is increased in acute liver failure (ALF). We have previously shown that ornithine phenylacetate (OP) attenuates ammonia rise and intracranial pressure in pigs suffering from ALF, but failed to demonstrate a stoichiometric relationship between change in plasma ammonia levels and excretion of phenylacetylglutamine in urine. The aim was to investigate the impact of OP treatment on the phenylacetylglutamine pathway as an additional ammonia-lowering pathway.

Methods: A well-validated and characterized large porcine model of ALF (paracaval anastomosis followed by hepatic artery ligation), which recapitulates the cardinal features of human ALF was used. Twenty-four female pigs were randomised into three groups: (i) Sham operated + vehicle, (ii) ALF + vehicle, and (iii) ALF + OP.

Results: There was a significant increase in arterial glycerol concentration in ALF (p<0.001 compared with sham), with a 3-fold increase in glycine release into the systemic circulation from the kidney compared to the sham group. This increase was attenuated in both blood and the brain of the OP treated animals (p<0.001 and p<0.05, respectively), and the attenuation was associated with renal removal of glycine through excretion of the conjugation product phenylacetylglutamine (PAG). ALF+vehicle: 1000 ± 106 μmol/L, ALF+OP: 2762 ± 2670 μmol/L, p<0.003.

Conclusion: Data from this study provides solid evidence for the existence of a novel, and additional pathway for ammonia removal in ALF, that involves glycine production and removal, which is targeted by OP.

O10
Elevated serum bile acids contribute to hepatic encephalopathy progression by activating FXR signaling
McMillin M, Frampton G, Galindo C, DeMorrow S

Background: Hepatic bile acids are synthesized in the liver from cholesterol via the enzyme Cyp7a1 and are normally reabsorbed by the liver following reuptake from the enterohepatic circulation. Following liver injury, hepatic bile acid reabsorption is disrupted leading to elevated serum bile acid levels. Acute liver failure leads to loss of liver function with subsequent systemic complications including hepatic encephalopathy (HE). During normal states, the brain uses Cyp27a1 and Cyp46a1 to metabolize cholesterol into bile acids in order to maintain cholesterol homeostasis. Besides their lytic properties, bile acids can act as signaling mediators via the nuclear receptor, farsenoid x receptor (FXR) which leads to feedback inhibition of bile acid synthesis. We hypothesize that during HE, elevated bile acids worsen neurological outcomes by activating FXR signaling and increased levels of nuclear cholesterol.

Methods: Male C57Bl/6 mice and mice with reduced hepatic bile acid synthesis, Cyp7a1-/- mice, were given an intraperitoneal injection of 100 mg/kg azoxymethane (AOM) to induce liver failure and were monitored for neurological decline. Bile acids and cholesterol in the circulation and/or brain were measured using commercially available kits. In the brain, expression of apical sodium-dependent bile acid transporter (ASBT), FXR, Cyp27a1 and Cyp46a1 were assessed via RTPCR and immunoblotting. Neural FXR protein was reduced via intracerebroventricular infusion of FXR Vivo-morpholino sequences (1 mg/kg/day for 3 days). Results: AOM mice had elevations of both circulating and cranial bile acid levels, which did not occur in Cyp7a1-/- mice that were protected from neurological decline. ASBT and FXR were elevated in the cortex of AOM mice, an effect not observed in Cyp7a1-/- mice. FXR Vivo-morpholino infusion protected AOM mice from neurological decline. AOM mice had reduced activity of Cyp27a1 and Cyp46a1, an effect not observed in FXR Vivo-morpholino infused AOM mice. Also, cortical cholesterol levels in AOM mice were elevated and were significantly reduced in FXR Vivo-morpholino-treated mice. Conclusions: Liver failure increases circulating bile acids, which activate FXR signaling, and worsen neurological decline. Elevated cortical cholesterol, due to activated FXR signaling, exacerbates HE pathology. This data supports that targeting bile acid synthesis or FXR signaling in the brain may be effective treatments for patients with HE.

O11
INCREASED 3-NITROTYROSINE IS ASSOCIATED WITH REDUCED WHITE MATTER MICROSTRUCTURAL INTEGRITY AND COGNITIVE DEFICITS IN MINIMAL HEPATIC ENCEPHALOPATHY
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Background & aims: Cirrhotic patients with minimal hepatic encephalopathy (MHE) show mild cognitive impairment and psychomotor slowing. Magnetic resonance studies using diffusion tensor imaging (DTI) suggest that these patients may show white matter abnormalities. This work aims were 1) to investigate whole-brain white matter microstructural abnormalities associated with MHE using TBSS; 2) to evaluate the correlations of white matter microstructure changes with performance in psychometric test evaluating different cognitive and motor coordination deficits; 3) assess whether some peripheral biomarker correlates with changes in microstructural integrity of white matter tracts.

Methods: White matter microstructure integrity was analyzed using DTI imaging and Tract-Based Spatial Statistics (TBSS) in 17 controls, 15 cirrhotics without and 15 with MHE. Psychometric tests assessing different functions were performed and several biochemical parameters were measured in blood.

Results: Patients with MHE (but not without MHE) show reduced overall white matter structural integrity, with increased mean diffusivity (MD) and reduced fractional anisotropy (FA). Reduced FA of some tracts correlate with performance in line tracing and serial dotting tests. Increased MD correlate with performance in these same tests and in the ymbol digit and number connection A tests and with serum levels of 3-nitrotyrosine. These findings suggest an association between microstructural alterations and reduced performance in attention, mental processing speed, visuospatial and visuomotor coordination tests.

Conclusions: Analysis of white matter microstructural integrity by DTI may provide new, strong, in vivo neuroimaging biomarkers for early diagnosis of MHE and to follow the efficacy of treatments.

O12
Hyperammonemic rats show expression changes in neurodegeneration related genes.
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Background and Aim: The neurodegeneration process is a slow, progressive loss of synapses and neurons in the central nervous system, which determines the onset of cognitive...
and motor disorders. Traditionally, hepatic encephalopathy (HE) is a metabolic disorder that affects astrocytes, but respects neuronal architecture. However, a neuronal loss seems to be present in HE, frequently associated with repeated or prolonged episodes of HE and portosystemic anastomosis (PCA). The aim of the study was to identify the elements that contribute to neurodegeneration in a new experimental model of episodic HE and assess the relationship between nitrogen metabolism, inflammation and neurodegeneration by means of a differential expression analysis.

**Methods:**
A simulation of repeated and prolonged episodes of HE was induced in SD-OFA male rats (200g) for six months by PCA and ammonium acetate infusion (55mM/Kg·min during 3 hours). A group of animals was also infused with lipopolysaccharides (LPS; 3mg/Kg) to induce inflammation. The gene expression was assessed in the cortex and cerebellum using an Affymetrix® Rat Gene Array 1.1. The study was performed on PCA rats with hyperammonemia (n=5), inflammation (n=5) and both hyperammonemia and inflammation rats (n=4) and was compared to saline-infused sham rats (n=5) and saline-infused PCA rats (n=5).

**Results:**
Compared to saline-infused sham rats, LPS group showed an increase in inflammation related genes such as C3, CXCL13 and LCN2 that promote neuronal apoptosis by microglia activation (Fold Change: Cortex = 3.3, Cerebellum = 3.4). Ammonia group showed mainly a down regulation in cortex genes related to neurodegenerative disorders such as Adora2A and a clear up regulation in cerebellum of transtiretin (TTR), a protein associated to amyloidosis in humans (Fold Change TTR = 17.4). The array results were validated by Real-Time PCR.

**Conclusion:**
Gene experiments demonstrate evidences of neurodegeneration in a HE animal model. These expression changes suggest that ammonia overdose is able to affect motor functions via neuronal impairment in the central nervous system. The important over expression of TTR in cerebellum requires further investigation.

**O13**
**Hepatic and Renal expression of the ammonia transporter Rhesus protein (Rhcg) plays a critical role in modulating hyperammonemia and hepatic encephalopathy in liver failure**

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**Introduction:**
Ammonia is central in the pathogenesis of hepatic encephalopathy (HE). Although ammonia gas (1-2%) can diffuse readily across the plasma membrane, ammonium ions (98%) need to be transported into cells for metabolism. Rhcg is a recently described ammonia transporter, transmembrane protein that is expressed in the kidneys and involved in modulating acid-base balance. Its expression in the liver disease and brain is unknown. This study was designed to determine the role of this protein in mediating hyperammonemia and hepatic encephalopathy in liver failure.

**Materials and Methods:**
Three studies were performed. Study 1: Gene and protein expression of Rhcg was determined in the liver, kidney and the brain of sham operated (Sham, n=6) and bile duct ligated (BDL, n=6) rats. Study 2. Induced hyperammonemia study: Wild type (WT, n=10), and Rhcg knock-out mice (Rhcg-KO, n=11) were treated with NH4Cl or saline (0.2 M) for 3-days in the drinking water after which they were sacrificed and their plasma ammonia and brain water measured. Study 3. Induction of acute liver failure (ALF): WT (n=7) and the Rhcg-KO (n=7) animals were administered 500mg/Kg acetaminophen (APAP) or saline, IP. Animals were sacrificed at coma stages and plasma ammonia and brain water were measured.

**Results:**
Study 1. Plasma ammonia and brain water was significantly higher in BDL animals compared with the Sham animals (p<0.01 each). This was associated with significantly greater gene and protein expression of Rhcg in the liver (7-fold) and kidneys (2-fold) but not the brain. Study 2. Basal levels of ammonia in the Rhcg-KO animals were significantly higher than the WT animals (p<0.01). The WT groups maintained the value after induction of hyperammonemia (p<0.01). Study 3. Arterial ammonia was significantly higher in the ALF animals compared with controls (p<0.0002), which was higher in the Rhcg-KO animals compared with WT group and this was associated with reduced time to coma stages (p=0.05) and significantly greater brain water (p=0.01) in the Rhcg-KO animals.

**Conclusion:**
The results of this study show for the first time, a critical role for the hepatic and renal expression of the ammonia transporter, the Rhcg protein, as a mechanism that regulates ammonia levels in health, during hyperammonemia and liver failure. Further understanding the mechanisms of its regulation may provide new approaches to treating hepatic encephalopathy.
ABSTRACTS

expression of dimethylarginine-dimethylaminohydrolase-1 (p<0.05) [DDAH 1- responsible for metabolism of ADMA], whilst also significantly lowering hepatic inflammatory genes such as phosphorylated NFkB, iNOS, 4HNE and 3-nitrotyrosine expressions, compared with placebo treatment.

Conclusion. Our study is the first indication that there is an association between hyperammonemia and portal hypertension in cirrhosis. Treatment of hyperammonemia with ornithine phenylacetate reduces hepatic inflammatory mediators and thus the severity of portal hypertension in a clinically relevant model of cirrhosis. We observed restoration of the hepatic eNOS activity, and expression of the eNOS regulators, DDAH1/ADMA and caveolin-1. Thus, our data provide the rationale for evaluating ornithine phenylacetate in the treatment of hyperammonemia induced hepatic injury and portal hypertension. Key words: eNOS dysfunction, Hyperammonemia, Hepatic Inflammation, Ornithine Phenylacetate, Portal hypertension.

O15 NEGATIVE PROGNOSTIC IMPACT OF HEPATIC ENCEPHALOPATHY IN CIRRHOSIS: IS TIME TO INTEGRATE THE MELD SCORE TO IMPROVE THE TRANSPLANT BENEFIT?

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Background and aims: Even though is well known that hepatic encephalopathy (HE) is associated with poor outcome in cirrhotic patients, the current liver graft allocation policy does not consider this burden. The aim of the present study was to analyze if HE may confer additional mortality risk beyond MELD.

Methods: We included outpatients consecutively listed for liver transplantation (LT) from 2002 to 2013 and consecutive hospitalized patients from 2008 to 2013. Patients older than 70 years, HCC outside Milan criteria or TIPS were excluded. Patients were considered as “HE+” in case of previous or actual hospitalization for HE. The follow-up was until LT, death or TIPS placement. AUCROC and net reclassification index (NRI) were used to assess improvements in mortality prediction after the addition of HE.

Results: 486 patients were included (300 outpatients and 175 inpatients). The group of “HE+” patients presented a more severe liver injury with a higher MELD and MELD-Na scores (p<0.001), lower albumin level (p<0.001) and more episode of ascites (p<0.001). During the first 6 months 77 patients died, 50 were transplanted and 6 underwent to TIPS. At univariate analysis, MELD, “HE+” (p<0.001), serum sodium (p<0.001), ascites (p<0.003) and an history of hospitalization for any causes (p=0.03) were associated to a higher 3 and 6-months mortality. At multivariate analysis, MELD (HR 1.16; IC: 1.1-1.22; p=0.0001), sodium (HR 0.89; 0.85-0.94; p<0.0001), “HE+” (HR 3.6; 1.8-7.1; p=0.0002), but not hospitalization, were independent mortality predictors. Excluding patients with HCC, the AUCROC of the MELD-HE score was significantly higher than MELD alone (0.79 vs 0.76; p=0.04) in predicting 6-months mortality, with a NRI of 0.24 (p<0.01). Including patients with HCC (considered as MELD 22), the AUCROC for the MELD-HE score was also significantly higher than MELD (0.75 vs 0.67, p<0.02), with a NRI of 0.2 (p<0.01).

Conclusions: HE increases 4-folds the 6-months mortality and improves significantly the predictive value of MELD. The integration of MELD with HE may improve transplant allocation policy.

O16 BENEFICIAL EFFECTS OF MUSCLE MASS OPTIMIZATION USING LEUCINE SUPPLEMENTATION AND EXERCISE TRAINING IN THE PREVENTION OF HEPATIC ENCEPHALOPATHY IN EXPERIMENTAL CIRRHOSIS

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Background: Malnutrition is an important prognostic factor potentially influencing clinical outcome of patients suffering from chronic liver disease (cirrhosis; CLD). Malnutrition, considered a consequence of metabolic disturbances (hypermetabolism), exacerbates severe muscle loss and hepatic encephalopathy (complex neuropsychiatric disorder) in cirrhotic patients. Nutritional therapies, which focus on muscle mass and circumference and metabolic activity, which was further ameliorated with EX. In addition, BDLS rats receiving LEU and EX exhibited less anxiety-like behavior as well as better novel object recognition memory.

Conclusion: Our results demonstrate that supplemental LEU along with EX reduces body weight and muscle mass loss, improves metabolic activity, attenuates brain edema and improve cognitive and psychomotor function. These findings suggest that strategies aiming at improving nutritional status will attenuate muscle mass loss, reduce the risk of developing hepatic encephalopathy and therefore improve quality of life and decrease mortality risk in CLD. LEU supplementation and EX could rapidly be translated into clinical practice.

O17 BACTERIAL ANTIGEN TRANSLLOCATION IN PATIENTS WITH CIRRHOSIS AND MINIMAL HEPATIC ENCEPHALOPATHY IS ASSOCIATED WITH INCREASED SERUM AMMONIA AND NITRIC OXIDE LEVELS.

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Background & Aims: Bacterial translocation is a frequent event in patients with cirrhosis and ascitic fluid. Minimal hepatic encephalopathy (MHE) is also frequent among patients with cirrhosis. Several enteric bacteria are natural producers of ammonia. We investigated whether an association between bacterial products translocation and serum ammonia levels can be established in decompensated cirrhotic patients with MHE.

Patients & Methods: Consecutively admitted cirrhotic patients with ascites and MHE from Hospital General Universitario de Alicante, Spain, and Hospital Valme de Sevilla, Spain, were included. Clinical and alytical data were recorded. MHE was diagnosed by using the psychometric hepatic encephalopathy score (PHES). Serum bacterial DNA, endotoxin, ammonia and nitric oxide levels were measured.

Results: Forty patients were included in the study (29 male, mean age 60±9.8 years). Etiology was mainly alcohol (n=22, 55%) and HCV (n=11, 27.5%). Fifteen patients (37.5%) presented ascites. Mean MELD score was 10.9±4.6 and mean INR was 1.30±0.24. Bacterial DNA was identified in 12 patients (30%) and corresponded to Gram-negative microorganisms in 10 cases. No differences were observed in any clinical or analytical variable when patients were not treated by the presence of bacterial DNA: Serum levels of ammonia (2.30±1.92 vs 5.5±1.80 nmol/L), endotoxin (1.7±1.65 vs 4.40±0.77 UE/mL) and nitric oxide (15.10±2.54 vs 26.50±2.60 nmol/mL) were significantly higher in patients with blood bacterial DNA translocation (p<0.01 in all cases). Mean amplified bacterial DNA concentration was (25±3.9 ng/ul).

Serum ammonia levels showed a positive correlation with endotoxin levels (r=0.74, p<0.01) and with nitric oxide levels (r=0.72, p<0.01) in the overall series of patients. Among patients with bacterial DNA, serum ammonia levels showed a positive correlation with amplified bacterial DNA concentration (r=0.78, p<0.001).

The median PHES score was significantly higher in patients with bacterial DNA translocation (6 [12 - 4] vs 8 [11 - 9], p=0.03).

Conclusions: Bacterial antigenic product translocation into blood in cirrhotic patients with minimal hepatic encephalopathy is associated with significantly increased serum ammonia and nitric oxide levels.

Methods: CLD was induced in rats following 6-week bile duct ligation (BDL). Five experimental groups were tested; 1) BDL; 2) BDL + LEU; 3) BDL + EX; 4) BDL + LEU + EX; 5) Sham-operated rats. One week following BDL, rats were submitted to 15 min EX (10 cm/s) every other day and BDL rats receiving LEU were gavaged daily (1.35 mg/kg) for 5 weeks. Body weight, muscle (gastrocnemius) mass, metabolic state (calculation of energy expenditure independent of food intake and fecal mass), cerebral edema (specific gravity method) and cognitive/psychomotor function (open-field test; anxiety-like behavior assessment and novel object recognition test; memory testing) were measured in all groups.

Results: BDL rats gained less body weight and muscle mass compared to sham-operated rats. LEU-treated BDL rats display an improvement in brain edema, muscle mass and circumference and metabolic activity, which was further ameliorated with EX. In addition, BDL rats receiving LEU and EX exhibited less anxiety-like behavior as well as better novel object recognition memory.

Conclusion: Our results demonstrate that supplemental LEU along with EX reduces body weight and muscle mass loss, improves metabolic activity, attenuates brain edema and improve cognitive and psychomotor function. These findings suggest that strategies aiming at improving nutritional status will attenuate muscle mass loss, reduce the risk of developing hepatic encephalopathy and therefore improve quality of life and decrease mortality risk in CLD. LEU supplementation and EX could rapidly be translated into clinical practice.
Hepatic Encephalopathy (HE) in Paracetamol-induced Acute Liver Failure (PALF) is associated with IL-8 and ammonia-induced neutrophil TLR9 expression culminating in neutrophil exhaustion.

Methods: Neutrophil TLR9 expression and cytokine production were studied ex-vivo at baseline and in response to stimulation with lipopolysaccharide (LPS) (200ng/ml) and ammonium chloride (400µM). NPA and OB were measured by determining their ability to ingest opsonised bacteria (E. coli) and produce reactive oxygen species, respectively.

Results: NPA was decreased and OB increased in patients with PALF and AAH on day 1 compared to HC (p<0.0001), whereas TLR4 were decreased in PALF and AAH on day 1 compared to HC (p<0.0001), whereas TLR9 expression was increased in PALF on day 1, compared to AAH and HC (p<0.0001). Arterial ammonia was higher in PALF than AAH (p<0.005). Neutrophil TLR9 expression correlated with plasma IL-8 and peak ammonia concentrations (r²=0.6; p<0.05) and increased with severity of HE (0-2 vs 3/4) and SIRS score (0-1 vs 2-4) (p<0.05). Those patients with advanced HE (grade 3/4) or high SIRS score (2-4) on day 1 had elevated neutrophil TLR9 expression and higher ammonia and plasma IL-8 culminating in neutrophil exhaustion, with neutrophils failing to produce pro-inflammatory cytokines upon challenge. Healthy neutrophil TLR9 expression could be induced upon co-stimulation with IL-8 and ammonia but not independently.

Conclusion: These data point to neutrophils being influenced by ammonia and IL-8 in a synergistic fashion in PALF inducing systemic inflammation, neutrophil exhaustion and influencing the severity of HE.
Christopher Rose
The novel glutaminase inhibitor CB-839 prevents oral glutamine-induced hyperammonemia in portacaval shunted rats
Cristina R. Bosoi, Mélanie Tremblay, Christopher F. Rose
Hepato-Neuro Laboratory, CRCHUM, Université de Montréal, Canada.

Background: Ammonia plays a major role in the pathogenesis of hepatic encephalopathy (HE) and therefore ammonia-lowering treatments remain a primary therapeutic strategy. Glutamine deamidation by the mitochondrial enzyme glutaminase (GLS) is believed to a major source of ammonia production in cirrhotic patients and increased intestinal GLS activity has been shown to be linked to minimal HE and to an increased risk of developing overt HE. CB-839 is a potent, selective and orally bioavailable GLS inhibitor (Gross et al., Mol Cancer Ther 13:890) that is currently in Phase 1 clinical trials for the treatment of cancer (clinicaltrials.gov). Aim: To evaluate the effect of CB-839, a GLS inhibitor, in preventing the onset of hyperammonemia following an oral glutamine challenge (OGC) in rats with portacaval anastomosis (PCA).

Methods: Four week PCA rats received a single dose of CB-839 (gavage, 200 mg/kg diluted in 5ml/kg of vehicle). Control PCA rats received equivalent volumes of vehicle. Four hours after CB-839 or vehicle administration, PCA rats received an oral glutamine challenge (gavage, 100 mg/kg). Repeated aortic blood samples were obtained at baseline, 0.5h, 1h, 1.5h, 2.5h, 2.5h, 3h and 4h following OGC. Glutamine and ammonia were measured using commercial available kits.

Results: Baseline ammonia levels were similar in both PCA groups. Following OGC, blood ammonia increased in vehicle-treated PCA rats with a peak at 2h (2.5-fold increase vs baseline, p=0.05). In CB-839 treated-PCA rats, ammonia levels did not change compared to the baseline value and were significantly decreased compared to non-treated PCA rats (p<0.05). At 4h, ammonia levels returned to baseline values in both groups. Baseline glutamine levels were not significantly different between treated and non-treated PCA rats. Following OGC, no significant difference between glutamine levels was observed in non-treated PCA rats compared to baseline values. However, in PCA rats treated with CB-839 glutamine levels significantly increased compared to non-treated PCA controls attaining a peak at 2.5 h (1.59 ± 0.40 mM vs 0.60 ± 0.15 mM, p<0.05). At 4h, glutamine levels remained significantly increased. Conclusions: CB-839 treatment inhibited glutamine induced hyperammonemia in PCA rats. These preliminary results strongly suggest CB-839 is an effective agent to attenuate GLS-induced ammonia production. Further studies are warranted to evaluate CB-839 as a novel agent for the treatment of HE.

Alexander Thrane
A critical reappraisal of astrocyte volume regulation and interstitial fluid dynamics in brain edema formation and resorption
Alexander S. Thrane1,2,3, Vinita Rangroo Thrane1,2,3 and Maiken Nedergaard1

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Brain edema formation can complicate a range of neurological conditions, including infarction, tumors, trauma and metabolic encephalopathy. Although brain edema is associated with significant morbidity and mortality, contemporary therapeutic options are often only partly effective and many remain incompletely understood. Recent studies have revealed the existence of a brain-wide paravascular pathway, termed the ‘glymphatic system’, for cerebrospinal (CSF) and interstitial fluid (ISF) exchange. The current talk aims to critically re-examine astrocyte volume regulation and interstitial fluid exchange with regard to the main types of brain edema, with a particular emphasis on metabolic encephalopathies such as hepatic encephalopathy (HE). Our results suggest that in cytotoxic edema, energy depletion may cause astroglial swelling to become localized to a small central zone in injured, infarcted or metabolically compromised areas. Conversely, most fluid influx may occur in the surrounding, better-perfused tissues where enhanced glymphatic CSF influx and suppressed ISF efflux forces an expansion of the interstitial compartment. Astrocyte volume regulation plays a critical part in this phenomenon; as in vivo studies indicate that astroglia are capable of tightly regulating cell volume to a broad range of challenges so long as there is adequate energy supply. Ours and other recent studies also promote the idea that paravascular inflammation plays a critical role in the vasogenic edema that often accumulates later in the course of brain disorders. Finally, the talk will briefly discuss how recent advances in diagnostic imaging of glymphatic function may help to better define the edema profile of individual patients and enable more specific therapy.
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