TREATMENT STRATEGIES
HEPATOLOGY

Volume 1 Issue 1

- Gallstones
- Gut Microbiota
- Hepatic Encephalopathy
- Hepatitis
- Hepatocellular Carcinoma
- Insulin Resistance
- Liver Diseases
- Liver Metastasis
- Pancreatic Diseases
- Transplantation

EASL
EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER

Includes a Review of the 49th Annual Meeting of the EASL - ILC 2014

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TREATMENT STRATEGIES - HEPATOLOGY

TREATMENT STRATEGIES - HEPATOLOGY - May 2014

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Welcome...

I am delighted to welcome you to the inaugural edition of *Treatment Strategies - Hepatology*. In this edition we bring you a range of informative articles, papers and reports, as well as an in-depth review of the 49th Annual Meeting of the European Association for the Study of the Liver (EASL) - The International Liver Congress™ (ILC) 2014, which was held in London in April. Our ILC 2014 congress review will provide you with the breaking news, research highlights and the best of the symposia, as well as a number of poster synopses. We really feel that this review will provide you with the not-to-be missed highlights of the congress.

This edition also features a number of interesting and informative papers on subjects such as gallstones, gut microbiota, hepatic encephalopathy, hepatitis, hepatocellular carcinoma, insulin resistance, liver diseases, liver metastasis, pancreatic diseases and transplantation. With these papers we aim to bring you new insights into the latest treatment strategies for a number of conditions and diseases, and we hope that you enjoy this carefully chosen content.

So far, 2014 is proving to be a fantastic year for The Cambridge Research Centre, with some exciting changes including Treatment Strategies TV, where you can find footage from the most important scientific conferences, meetings and congresses, as well as interviews, symposia proceedings, roundtable events and much more. We also launched our range of interactive eBooks on iBooks, which is a great new way to read and download our content to your devices. Have you liked our new Facebook page? Here you can find all of the latest news about new projects and upcoming releases, and the Treatment Strategies' team are also all active on Twitter and LinkedIn.

We hope that you enjoy this edition of *Treatment Strategies – Hepatology*, and please do share your thoughts with us on this issue as well as what you would like to see in our next edition, which will feature a review of ILC 2015 - See you in Vienna.

Nigel Lloyd, Managing Director

The Cambridge Research Centre wishes to thank the following societies for their assistance and involvement in this publication.

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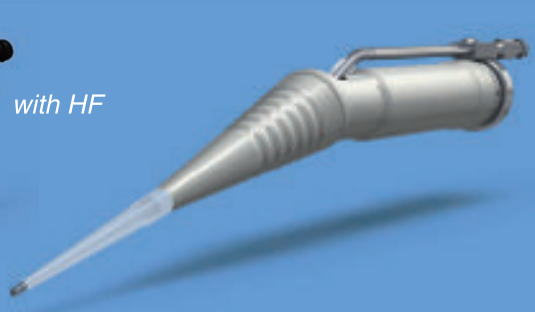

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CONTENTS...

CHRONIC LIVER DISEASE

A SLOW, SILENT, BUT PREVENTABLE DEATH

According to the World Health Organization, in 2008, 47.147 Europeans died of liver cancer, and 84.697 Europeans died of liver cirrhosis. These figures combined exceeded breast cancer mortality figures (103,255)[1].

Worldwide, both cirrhosis and liver cancer are on the rise.

THE CAUSES

The major causes of chronic liver disease are:

- Lifestyle (alcohol and overweight/obesity, leading to alcohol- or non-alcohol related fatty liver disease)
- Virus infection (mainly viral hepatitis B and C), and
- Genetic factors including autoimmune diseases

In addition, alcohol consumption by those who are infected with chronic hepatitis B and/or C multiplies the risk of developing cirrhosis and primary liver cancer[2].

ON THE POSITIVE NOTE, THE VAST MAJORITY OF CHRONIC LIVER DISEASE CASES CAN BE PREVENTED AND/OR TREATED

With the recent publication of its manifesto[3], ELPA calls on policymakers to ensure that an integrated approach, from prevention, to early diagnosis of a possible liver problem (e.g. via enzyme testing), to treatment, is taken to deal with the growing burden of liver disease.

ABOUT ELPA:

ELPA emerged from a desire among European liver patient groups to share their experiences. In June 2004, 13 patient groups from 10 European and Mediterranean Basin countries met to create the association. ELPA which formally launched in Paris, on April 14th 2005, now has 29 members from 24 countries.

ELPA and its members are dedicated to multi-level initiatives involving EU and national policymakers, liver specialist associations and public health experts.

European Liver Patients Association

ELPA

For more information about ELPA, please visit our website at **www.elpa-info.org** or contact:

Margaret Walker, CEO
Mobile number: +41 79 778 30 19
E-mail: margaret@elpa-info.org

[1]Blachier M, Leleu H, Peck-Radosavljevic M et al. *The Burden of Liver Disease in Europe: A Review of Available Epidemiological Data*. Geneva: EASL, 2013.
[2]Donato F, Tagger A, Gelatti U et al. *Alcohol and hepatocellular carcinoma: the effect of lifetime alcohol intake and hepatitis virus infections in men and women* Am J Epidemiol 2002, 155:323-331.
[3]http://www.elpa-info.org/elpa-news---reader/items/elpa-briefs-european-elections-candidates-on-chronic-liver-disease.htm

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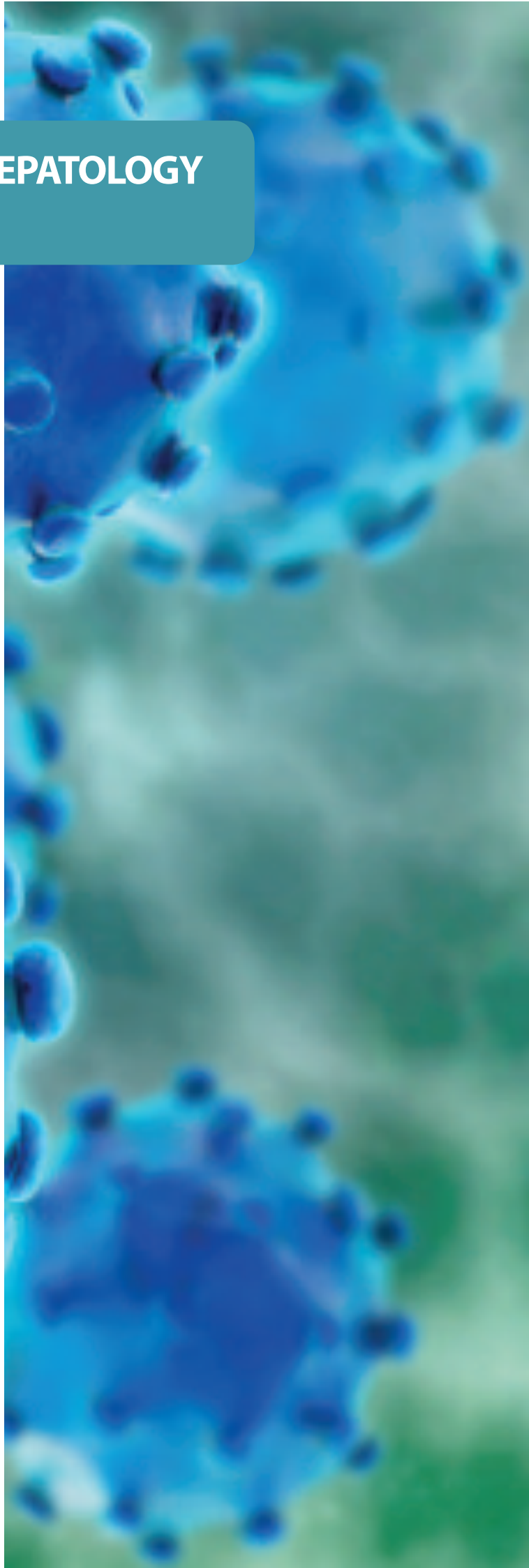


Foreword

Motaz Fathy Saad
Murabak Alkabir Hospital, Kuwait

Liver diseases have fascinated physicians since antiquity. However, the last decade witnessed logarithmically accelerated advances in our understanding of major causes of liver ailments, especially viral hepatitis. The molecular characterisation of hepatitis viruses A through E translated into a revolution in preventing and treating these infections. The dogma that chronic viral infections are incurable is challenged by the fact that chronic HCV is now curable in more than 90% of patients regardless of genotype thanks to the unraveling of the molecular mechanisms of viral replication, which in turn led to the design of a battery of direct acting agents that can block viral replication by inhibiting proteases and RNA polymerase. Not only is the cure rate impressive, but the side effects profile and safety of these medications are far superior to the conventional interferon plus ribavirin regimens.

Hepatitis B treatment has also seen great progress. The virus can be suppressed and cirrhosis can be prevented in most patients using highly potent drugs such as Entecavir and Tenofovir, both of which have a high genetic barrier for resistance.

Agents that can inhibit ccc DNA are expected to cure HBV and may become available in the not so distant future.

Hepatocellular carcinoma can be now treated using a number of interventions transarterial chemoembolisation (TACE), radiofrequency ablation, alcohol injection, surgical resection and liver transplantation. Pharmacotherapy using Tyrosine Kinase receptor antagonist Sorafenib improved survival and quality of life.

Another area of progress has been in the treatment of autoimmune hepatitis. Budesonide, a synthetic steroid with 90% hepatic clearance, is now licensed in some countries and has been shown to be very effective in inducing and maintaining remission with minimal side effects compared to conventional corticosteroids which have been the mainstay of treatment for decades.

Finding a reliable noninvasive technique of measuring hepatic fibrosis has been an illusive dream for hepatologists for years. Liver biopsy has been the gold standard in assessing liver histology. However, the procedure is invasive and associated with finite but definite risks. It is not suitable in patients with coagulopathy and ascites. Now a number of blood tests such as Fibrotest and Actitest have shown promising results. Imaging techniques such as MRI and Fibroscan have also become routine diagnostic tools in many centres with fairly good sensitivity and specificity for hepatic inflammation and fibrosis. Liver biopsy has now largely become a staging rather than a diagnostic tool for patients with equivocal results.

Despite this unprecedented progress, major challenges have emerged in the last decade especially in non-alcoholic fatty liver disease (NAFLD) linked to the worldwide epidemic of obesity. It is expected that NAFLD will be the leading indication for liver biopsy after a decade or so. New insights into the pathogenesis are expected to help design specific therapies to address insulin resistance as well as hepatic inflammation and fibrosis.

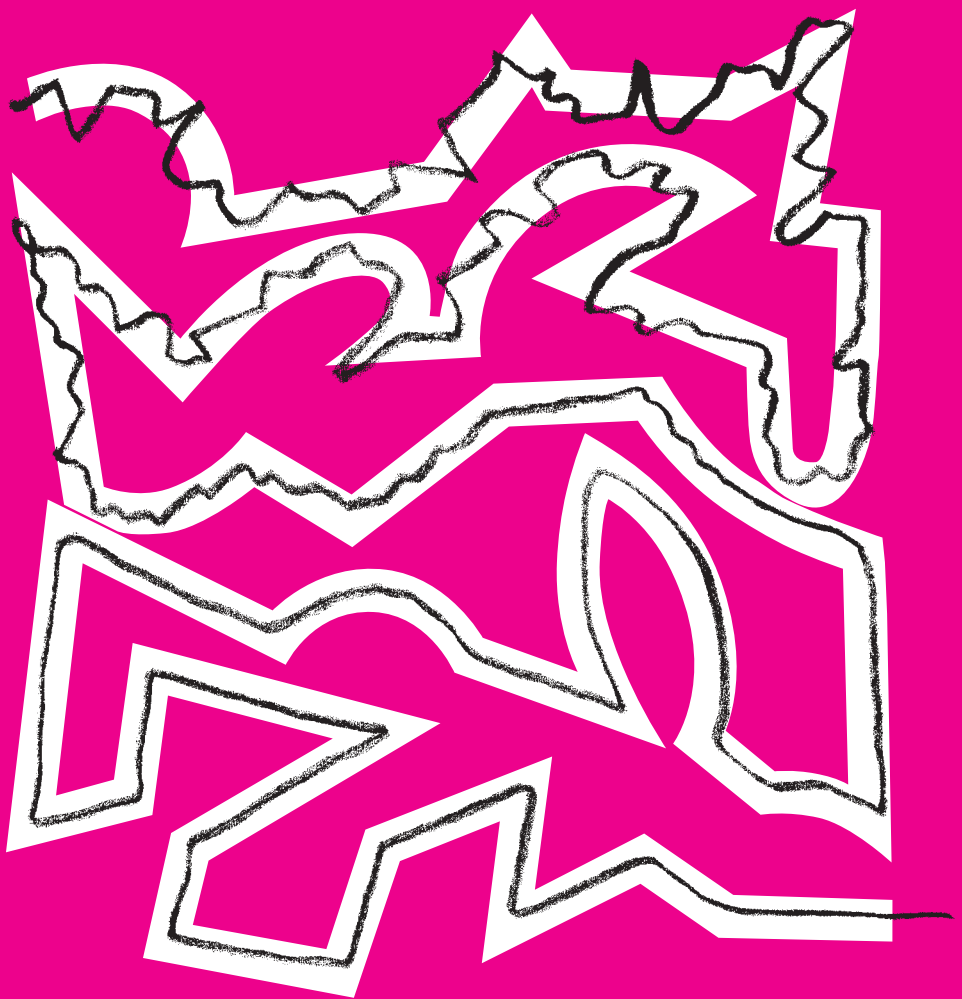
The enormous progress that has been made in liver transplantation over the past two decades has culminated in survival approaching 90% at 12 months. Nonetheless, the issue of availability of cadaveric organs remains an obstacle and many patients have to take the risk of staying on the waiting list for months. Resorting to living donors addressed this problem to an extent but alternatives such as hepatocyte transplantation, stem cell therapy and bioartificial liver support are being actively investigated.

It has been exhilarating to witness the tremendous progress in the management of liver diseases over the past decade. However, we are far from reaching the finish line. We need to keep the momentum to meet the ever-changing landscape of liver disease.

Motaz Fathy Saad, MSc, MRCP (UK), Speciality Certificate Gastroenterology (UK), graduated from Ain Shams Medical School in Cairo, Egypt, in 1998. He trained in the university's academic hospital, the Police Authority hospital and the International Medical Centre (IMC), Cairo, where he was a member of the team that established the liver transplantation unit with Japanese Surgeon Dr Koichi Tanaka. In 2007 he moved to Kuwait to work in Murabak Alkabir hospital, which is the academic hospital of Kuwait University in Haya Al-Habeeb GI centre, one of the two main gastroenterology and hepatology centres in the state, where he works as gastroenterologist and hepatologist with special interest in viral hepatitis.

Hepatic encephalopathy:

GET LIFE BACK ON TRACK



Bridging the gap between liver and brain: Forgetfulness, confusion, irritability, somnolence, tremor – hepatic encephalopathy is responsible for significant cognitive and physical dysfunction.[1] Hepa-Merz® stops the brain intoxication caused by liver cirrhosis, safeguarding the brain through a process of dual ammonia detoxification[1-4] – suitable for all stages of HE.[5]

Hepa-Merz® restores cognitive function to get patients' lives back on track.

Hepa-Merz® Granules. Active substance: L-ornithine-L-aspartate. **Composition:** One sachet with 5 g of Granules contains: **Active substance:** 3 g L-ornithine-L-aspartate. **Excipients:** citric acid, saccharin sodium, sodium cyclamate, povidone 25, fructose, flavorings, orange yellow S (E110). Note for diabetics: One sachet of Hepa-Merz® Granules contains 1.13 g of fructose (corresponds to approx. 0.11 BU). **Therapeutic indications:** Treatment of concomitant disease and sequelae due to impaired detoxification activity (e.g. in cirrhosis of the liver) with the symptoms of latent and manifest hepatic encephalopathy. **Contraindications:** Absolute: Hypersensitivity to L-ornithine-L-aspartate, orange yellow S or any of the other excipients. Severely impaired renal function (renal insufficiency). A serum creatinine value over 3 mg/100 ml can be used as a guideline value. Relative: Pregnancy and lactation: No clinical data are available relating to intake of Hepa-Merz® Granules during pregnancy. No exhaustive animal studies have been performed for L-ornithine-L-aspartate, to investigate its toxicity in relation to reproduction. Administration of Hepa-Merz® Granules during pregnancy should therefore be avoided. If, however, treatment with Hepa-Merz® Granules is considered necessary, careful consideration should be given to the benefit versus risk ratio. It is not known whether L-ornithine-L-aspartate is excreted into the breast milk. Administration of Hepa-Merz® Granules should therefore be avoided during lactation. If, however, treatment with Hepa-Merz® Granules is considered necessary, careful consideration should be given to the benefit versus risk ratio. **Undesirable effects:** Uncommon (≥ 1/1000 to < 1/100): Nausea, vomiting, stomach ache, flatulence, diarrhea. Very rare (< 1/10000): Pain in the limbs. These undesirable effects are usually transient and do not require withdrawal of the medicine. Orange yellow S (E110) can trigger allergic reactions. **Warnings:** Hepa-Merz® Granules contain fructose. Patients with rare hereditary problems of fructose intolerance should not take this medicine. **Further precautions:** As a result of the disease, the ability to drive and operate machinery may be impaired during treatment with L-ornithine-L-aspartate.

Hepa-Merz® Infusion concentrate. Active substance: L-ornithine-L-aspartate. **Composition:** One ampoule of 10 ml contains: **Active substance:** 5 g L-ornithine-L-aspartate. **Excipients:** Water for injections. **Therapeutic indications:** Latent and manifest hepatic encephalopathy. **Contraindications:** Absolute: Hypersensitivity to L-ornithine-L-aspartate. Severe renal impairment (renal failure). A serum creatinine level in excess of 3 mg/100 ml can be taken as a guide. Relative: Pregnancy and lactation: There are no clinical data available on the use of Hepa-Merz® Infusion concentrate in pregnancy. L-ornithine-L-aspartate has been investigated for reproduction toxicity only to a limited extent in experimental animal studies. The administration of Hepa-Merz® Infusion concentrate in pregnancy should therefore be avoided. If treatment with Hepa-Merz® is nevertheless thought to be necessary, the benefits and risks should be carefully assessed. It is not known whether L-ornithine-L-aspartate passes into breast milk. Administration of Hepa-Merz® should therefore be avoided during lactation. If treatment with Hepa-Merz® is nevertheless thought to be necessary, the benefits and risks should be carefully assessed. **Undesirable effects:** Uncommon (≥ 1/1000 to < 1/100): Nausea. Rare (≥ 1/10000 to < 1/1000): vomiting. Frequency not known (frequency cannot be estimated from the available data): hypersensitivity, anaphylactic reaction. Generally however, the gastrointestinal symptoms are transient, and do not necessitate discontinuation of treatment. They disappear on reduction of the dose or the infusion rate. **Further precautions:** Hepa-Merz® concentrate for solution for infusion can be mixed with the usual infusion solutions. So far no peculiarities have been observed with regard to miscibility. However, the ampoules should be admixed to the infusion solution only immediately before application. At high doses of Hepa-Merz® Infusion concentrate, serum and urine urea levels should be monitored. If liver function is substantially impaired, the infusion rate must be adjusted to the individual patient in order to prevent nausea and vomiting. Depending on the underlying disease, the ability to drive and operate machines may also be impaired on treatment with L-ornithine-L-aspartate. Hepa-Merz® Infusion concentrate must not be injected into an artery. Status: January 2013. Merz Pharmaceuticals GmbH, 60048 Frankfurt.

References: 1. Prakash R, Mullen KD. Mechanisms, diagnosis and management of hepatic encephalopathy. Nat Rev Gastroenterol Hepatol. 2010 Sep;7(9):515-25. **2.** Bai M et al. l-ornithine-l-aspartate for hepatic encephalopathy in patients with cirrhosis: a meta-analysis of randomized controlled trials. J Gastroenterol Hepatol. 2013;28(5):783-92. **3.** Butterworth RF. Pathophysiology of hepatic encephalopathy: a new look at ammonia. Metab Brain Dis. 2002;17(4):221-7. **4.** Toris GT et al. Hepatic encephalopathy: an updated approach from pathogenesis to treatment. Med Sci Monit. 2011;17(2):RA53-63. **5.** Hepa-Merz Summary of Product Characteristics. Merz Pharmaceuticals, 2013.

Hepa-Merz®
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International Liver Congress™ 2014

9 - 12 April, London

49th Annual Meeting of the European Association for the Study of the Liver

The Congress

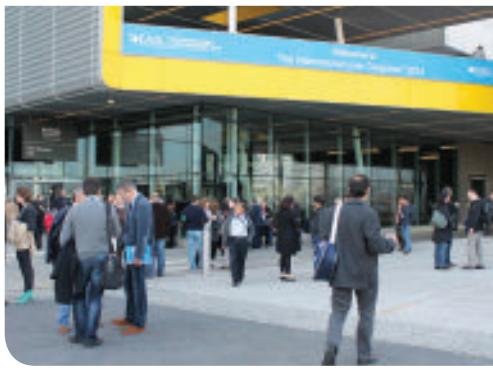
News from EASL ILC 2014

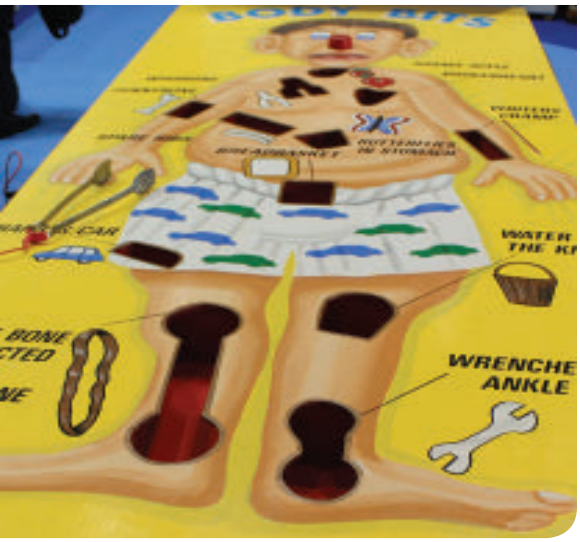
The association for the Study of the Liver (EASL) was founded in 1966 to promote and exchange knowledge in the field of liver diseases. Now the leading liver association in Europe, the EASL attracts the foremost hepatology experts as members and has an impressive track record in promoting research in liver disease, supporting wider education, and promoting changes in European liver policies.

Through out the year the EASL aims to work in continuing education in Hepatology with numerous events and educational initiatives, including:

- The International Liver Congress™
- EASL Monothematic
- EASL Special conferences
- EASL endorsed meetings
- Clinical & Basic Schools of Hepatology
- EASL Master Class
- EASL Fellowship programmes

This year the International Liver Congress™, the 49th annual meeting of the European Association for the Study of the Liver (EASL) was held in London's ExCel Centre between 9 -13 April 2014. Over ten thousand delegates took part in this years EASL conference, making 2014 the biggest EASL annual meeting to date.





To enhance the scientific and educational content of the congress the EASL Governing Board has maintained the general format of the congress whilst implementing some improvements. In doing so the International Liver Congress™ delivered an outstanding and attractive program for delegates this year, highlights include:

- PGC on Viral Hepatitis
- Clinical State-of-the-Art: Hormones and Obesity & Epigenetics
- 14 Joint Workshops with eminent Scientific organizations in the field of Liver Disease
- EU Session screening for Viral Hepatitis
- 3 basic, 1 translational, and 8 clinical symposia including topics like "Diagnosis & assessment of Liver Cancer", "HIV and the Liver", "Controversies in Liver Transplantation", and "Prevention and control of Viral Hepatitis"
- Late-breaking abstracts and Highlights in General Session 4

The 2014 revised recommendations on treatment of Hepatitis C were presented by Prof. Jean-Michel Pawlotsky at the International Liver Congress™ 2014. These EASL Clinical Practice Guidelines are intended to assist physicians and other healthcare providers, as well as patients and other interested individuals, by describing the optimal management of patients with acute and chronic HCV infections. Two protease inhibitors (PIs) have completed phase III development for patients infected with HCV genotype 1, and are currently registered for use in Europe and elsewhere. Therefore the EASL Clinical Practice Guidelines have been recently updated to include guidance on the use of these two drugs.

The International Liver Congress™ was also the chosen launch of the LiverTree™, the revolutionary online eLearning Portal of the European Association for the Study of the Liver. LiverTree™ is a one stop educational website featuring the most advanced search tool ever built in the field of liver research and assessed by liver experts. The most advanced eLearning portal ever built for a medical society, this centralizes and organizes thousands of EASL educational materials published over the past eight years in one central location. Including eLearning components such as accredited courses.

As well as a packed exhibition the ILC offered delegates numerous symposia at which last minute final results from

various studies were presented.

Gilead presented results for their fixed dose combination of sofosbuvir and ledipasvir with and without ribavirin (RBV), for the treatment of genotype 1 chronic hepatitis C virus (HCV) infection. Gilead reported the results from three phase 3 clinical studies announced topline results from three Phase 3 clinical trials (ION-1, ION-2 and ION-3) evaluating the investigational once-daily fixed-dose combination.

Abbvie reported new, detailed results from its hepatitis C development program at the International Liver Congress™ 2014. Data from TURQUOISE-II in adult patients with genotype 1 (GT1) chronic hepatitis C virus (HCV) infection with compensated liver cirrhosis was presented at the congress. Additionally, detailed results from the pivotal study, SAPPHIRE-I and SAPPHIRE-II, were also presented at the congress.

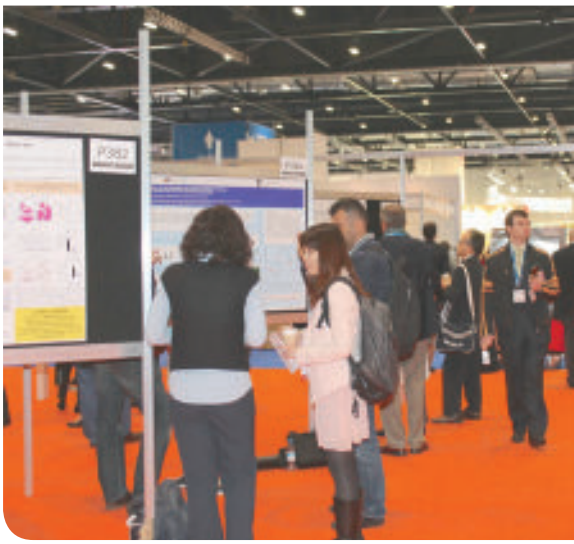
MSD announced additional data from the ongoing C-WORTHy study evaluating the efficacy and safety of a once-daily, all-oral regimen combining MK-5172, and MK-8742, among patients with chronic HCV Genotype 1 infection (GT1).

Bristol-Myers Squibb presented 12 abstracts at The International Liver Congress™. The key presentations included two sets of pivotal results from a global, Phase III study (HALLMARK DUAL) investigating the efficacy and safety of an all-oral, interferon- and ribavirin-free regimen of daclatasvir and asunaprevir.

Janssen presented the results from the Phase 2 COSMOS study demonstrating that 93 percent of patients with the hepatitis C virus and advanced liver fibrosis who were treated with simeprevir administered once daily with Gilead Sciences, Inc.'s sofosbuvir for 12 weeks achieved sustained virologic response 12 weeks after the end of treatment.

Sara Taheri, *Treatment Strategies*, is delighted to present our review of the International Liver CongressTM 2014 for the 49th Annual Meeting of the European Association for the Study of the Liver. This year the annual meeting was held in in London's ExCel Conference Centre from April 9 - 13, 2014.

Over the following pages, this review brings you the breaking news, research data, and symposia proceedings, as well as showcasing for you the latest drugs, products and devices pesented at the EASL - ILC 2014 congress.



EASL Meets in London

London is the capital and largest urban area of both England and the United Kingdom. An important settlement for two millennia, London`s history goes back to its founding by the Romans.

London is one of the world`s business, financial and cultural centres and its influence in politics, education, entertainment, media, fashion and the arts contribute to its status as a major global city.

The city is a major tourist destination both for domestic and overseas visitors, with annual expenditure by tourists of around £15 billion.

London is without a doubt, one of the liveliest and most visited cities in the world. This is a vibrant city filled with a mass of inspirational, nostalgia-inducing destinations attracting millions of international tourists each year.

Some of the major attractions of this city are: The Houses of Parliament, the Tower of London, The British Museum among others.

Recommendations for Managing Hepatitis C

During the International Liver Congress™ the EASL announced the new hepatitis C treatment guidelines (HCV).

These recommendations reflect the approval of three new direct-acting antivirals (DAAs) during 2014 by the European Medicines Agency. These three new HCV DAAs are more efficacious and better-tolerated and will be available on the European market in the first half of 2014, for use as part of combination therapies for HCV infection.

The new guidelines recommend that wherever possible, patients should be treated with the newest direct-acting antivirals.

EASL is also encouraging Physicians to 'mix-and-match' antivirals from different pharmaceutical companies to achieve the most potent interferon-free regimens, often in advance of full phase III trial data.

Recommendations are made for all genotypes, and include all direct-acting antivirals due to be licensed in Europe during 2014.

Sofosbuvir, a nucleotide analogue inhibitor of HCV ribonucleic acid (RNA)-dependent RNA polymerase, was approved in January 2014. Simeprevir, a second-wave, first-generation NS3/4A protease inhibitor will be approved in May 2014. Daclatasvir, an NS5A inhibitor, is likely to be approved in August or September 2014.

"Since EASL published the HCV Clinical Practice Guidelines in 2013, the treatment paradigm for HCV has changed with three additional direct-acting antivirals - sofosbuvir, simeprevir and daclatasvir –scheduled for approval this year for use in patients infected with HCV genotype 1", said Professor Jean-Michel Pawlotsky, Coordinator of the recommendations and Director of the French National Reference Centre for Viral Hepatitis.

Based on a systematic review of existing literature, the new recommendations provide best practice on a number of key areas:
- Indications for treatment: who should be treated?
- Available drugs (approved by EMA before the end of 2014)
- Treatment of chronic hepatitis C
- Treatment monitoring
- Measures to improve treatment adherence
- Post-treatment follow-up of patients who achieve an SVR
- Retreatment of non-sustained virological responders
- Treatment of patients with severe liver disease
- Treatment of special groups

The guidelines will be updated as soon as approval dates for new interferon-free combinations of sofosbuvir/ledipasvir (Gilead Sciences) and ABT-450/ritonavir, ombitasvir and dasabuvir (AbbVie) are known. These are likely to be approved in early 2015.

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Non-invasive Liver Monitoring

Dr Frank Neumann, CEO of Biaxxess, gave the Treatment Strategies team information on a non-invasive blood test used to diagnose and monitor different liver diseases.

One being the non-alcoholic liver disease, caused by inflammation which affects about 25 per cent of the adult population and has a high risk in progressing to liver cirrhosis or liver cancer.

Bioaxxes immunoassay can reliably diagnose and monitor this disease using just a drop of blood. This is due to the fact that the established conventional liver enzyme tests do not pick up this disease.

BIOAXXESS UK provides PEVIVA Cell Death Biomarker Assays to Pharma, Biotech, CRO and Clinical Research and Diagnostic Laboratory Services.

PEVIVA's first-in-class CE-certified IVD cell death biomarker assays M30-Apoptosense ELISA® and M65® ELISA, M65 EpiDeath® ELISA have been independently validated by pharmaceutical companies and clinical research centres for diagnostic use in oncology and hepatology.

M30-Apoptosense is an enzyme-linked immunosorbent assay (ELISA) for the quantitative detection of caspase-cleaved Keratin-18 (ccK18, K18F, K18Asp396-NE: M30 neo-epitope) levels in cytosolic cell extracts, cell culture supernatants, human serum or plasma or other body fluids. The M30-Apoptosense ELISA uses the M30 antibody which is specific to this K18 cleavage site and does not react with viable or necrotic cells.

The M30-Apoptosense ELISA measures the accumulation of a caspase-generated product resulting in an unmatched sensitivity thus allowing the tracing of apoptotic cells of epithelial origin in patient plasma/serum.

For more information please visit : **www.bioaxxess.co.uk**

Automatic and Continual Collects of Ascites with the Alfapump® System

Sequana Medical was delighted to attend this year's International Liver Congress in London and share the latest news and information on the alfapump® system.

The alfapump® system is the first and only system that automatically and continually collects ascites and moves it to the bladder, where it is passed naturally from the body.

The alfapump itself is a subcutaneously implanted battery powered pump that is charged through the skin. It ensures controlled and continual removal of ascites according to a programmed schedule set by the physician. Pressure sensors monitor pressure in the peritoneal cavity and bladder to ensure optimal fluid management. The alfapump can move up to four litres of ascites per charge.

The system has been uniquely designed to withstand the challenging conditions within the human body and is custom-engineered to provide robust, dependable and safe transport of ascites from the abdominal cavity to the bladder.

It is implanted subcutaneously using a minimally invasive procedure and the bladder and peritoneal catheters are placed using standard surgical techniques. They are then tunnelled to a small pump pocket located in the right upper abdominal quadrant where they are connected to the pump which is implanted under the skin.

In its clinical study, the alfapump system significantly reduced the median number of paracentesis procedures from 3.4 in the month prior to implant to 0.2 per month afterwards. This significant reduction in the need for paracentesis from once every 9 days to once every 127 days and in many cases no further paracentesis, is a huge benefit to patients, physicians and nursing staff alike.

With new DirectLink Technology patients and physicians are able to easily monitor the alfapump system is easily and conveniently. Each time the patient charges their alfapump, data from the pump are transferred to the handheld Smart Charger, once this is places in the docking station, the latest data are transferred securely via mobile network to alfapump data specialists which keep physicians -up-to date via data reports as often as required. As the alfapump is continuously monitored, patients can be sure that they are receiving optimal medical attention for the management of their ascites whilst reducing follow-up visits, and saving physicians valuable time.

For more information please visit **www.alfapump.com**

High Quality, Virological and Consultancy Services from Viroclinics Biosciences

Viroclinics Biosciences, a leading diagnostic and clinical trial operation service company had a strong presence during The International Liver Congress™. At the event they provided delegates with information on their high-quality, specific virological services and consultancy services were provided.

Viroclinics ensure timely delivery of results and perform services according to international guidelines endorsed by regulatory authorities under an ISO 15189 accredited quality system with elements of GCP and GLP, to comply with regulatory requirements and facilitate regulatory submissions.

Offering a full range of virology services for new drug development programs and post-marketing surveillance of existing drugs and vaccines, Viroclinics services range from traditional virology assays to the latest deep sequencing protocols for a very broad range of viruses.

With their BSL2 and BSL3 labs Viroclinics are able to perform analysis according to international ISO 15189 accreditation expanded with elements of GLP and GCP.

Through on-going collaborations with the Department of Viroscience of the Erasmus MC in Rotterdam, The Netherlands the company staff yields a strong, flourishing platform to act and implement assays at the forefront of Viroscience.

For more information visit: **www.viroclinics.com**

MARS the Leading Artificial Support Systems

Gambro, the leading supplier of artificial support systems, was showcasing the Molecular Adsorbent Recirculating System (MARS) for the treatment of patients with liver failure.

The MARS system is used in the treatment of liver failure to enable native liver regeneration by removing protein-bound and water-soluble toxins from the blood such as:

- ammonia
- urea
- aromatic aminoacids
- manganese
- benzodiazepin-like substances

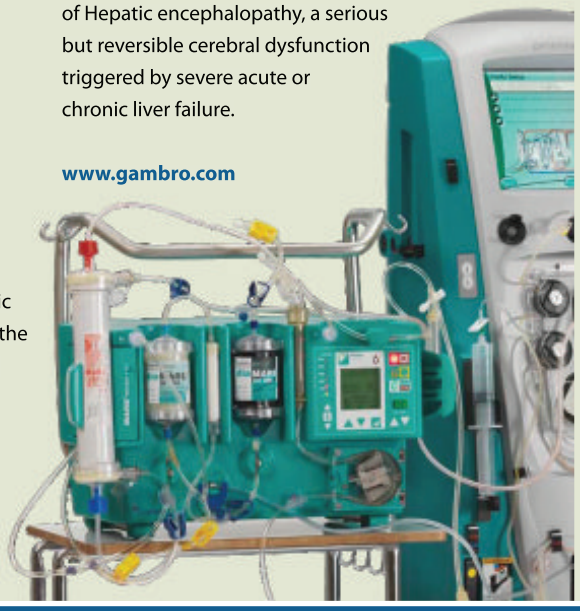
MARS removes protein-bound and water-soluble toxins with albumin dialysis. This reduces plasma toxicity, improves patient clinical conditions (hemodynamics, hepatic encephalopathy, urine output), enhances the regeneration of liver cells and may help to recover native liver functions.

The MARS system is compatible with several hemodialysis machines and CRRT devices and it can be added to patient's organ support, as it utilizes well-established equipment.

With early intervention the system can support the liver and help to prevent the evolution of multi organ failure contributing to an improved environment for hepatic regeneration and clinical recovery.

By reducing the toxins that reach the brain the MARS system prevents the development of Hepatic encephalopathy, a serious but reversible cerebral dysfunction triggered by severe acute or chronic liver failure.

www.gambro.com



Human Cell Based Bio-Artificial Liver Support System

Vital Therapies,® Inc. are a bio-therapeutic company focused on developing a cell-based therapy targeting the treatment of all forms of acute liver failure were showcasing the ELAD® System at the ILC 2014.

The ELAD® System is an investigational human cell-based bio-artificial liver support system that operates outside the body, or extracorporeal, and is designed with the intent to allow the patient's own liver to regenerate to a healthy state, or to stabilize the patient until transplant. The system incorporates human liver-derived cells, or VTL C3A cells, contained in four hollow fiber cartridges that are combined with single use customized disposable sets and a reusable ancillary delivery system.

During ELAD therapy, blood is drawn from the subject via a central venous line and then passes into the ancillary delivery system where plasma ultrafiltrate is isolated by an ultrafiltrate generator. The subject's plasma ultrafiltrate then passes into the four cartridges where it contacts our C3A cells after passing through fibers which allow appropriate two-way transfer of toxins, metabolites and nutrients, mimicking liver function. The fibers, made of a semi-permeable membrane, permit the passage of macromolecules and other substances from our C3A cells to the subject's plasma ultrafiltrate. At the same time, these fibers permit the passage of toxins such as bilirubin, and nutrients such as glucose, from the plasma ultrafiltrate to our C3A cells.

Treated plasma ultrafiltrate is then filtered, reconstituted with blood cells and returned to the subject via the central venous line. Meanwhile, the ancillary delivery system monitors temperature, pH, and oxygen concentrations in the plasma ultrafiltrate in order to maintain the cells' viability.

Data from ELAD clinical studies has shown trends that may indicate a potential to increase survival rates in patients with acute liver failure. ELAD has received orphan designation in the United States and Europe for the treatment of patients with acute liver failure.

The ELAD System therapy is not patient specific and our VTL C3A cells, which are derived from a single source, are used to treat all patients (allogeneic cellular therapy). In contrast, autologous cellular therapy uses a patient's own cells, which are manipulated in individual production batches, and is a costly and complex process. As a result, the production and logistics of treatment with our VTL C3A cells does not face the challenges commonly associated with autologous cellular therapies.

Unlike other potential therapies developed for acute liver failure there are significant combination of attributes that make the ELAD System unique these include:

- ELAD is a biologically active system that is designed to replicate many liver functions.
- The ELAD System is based on human cells which confer a considerable advantage over non-human animal-based cell therapies. Since humans possess naturally occurring antibodies, the use of human cellular therapy presents lower immunological risk compared to non-human animal tissues presents greater immunological risk compared to human cellular therapy. Additionally repeated treatments with a porcine cell may cause subsequent immune responses to become increasingly severe.
- The VTL C3A cells used in the ELAD System are derived from a human hepatoblastoma and are immortal. They can be expanded and can survive a session of up to ten days of ELAD System therapy, usually without needing to be replaced during treatment.
- Commercially scalable.
- ELAD cartridges are put into a dormant state and can survive up to 60 hours before being used for treatment therefore minimal manipulation needed by site.

ELAD has received orphan designation in the United States and Europe for the treatment of patients with acute liver failure.

For more infomration visit: **www.vitaltherapies.com**

Impeccable Images with MultiWave™ Technology

SuperSonic Imagine was presenting its range of leading ultrasound medical imaging devices. A team of experts and research scientists who specialise in the fields of ultrasound and/or medical imaging, SuperSonic Imagine is dedicated to developing innovative ultrasound systems including the Aixplorer®.

SuperSonic Imagine's innovative ultrasound architecture called SonicSoftware™ uses the latest generation multi-core processors along with the most advanced technology from the graphic game industry to shift ultrasound processing from hardware to software, thus enhancing speed, accuracy and flexibility. SonicSoftware enables other innovations such as MultiWave™ Technology.

This revolutionary system is the only system that images two different kinds of waves to better characterise tissue with MultiWave™ technology with:

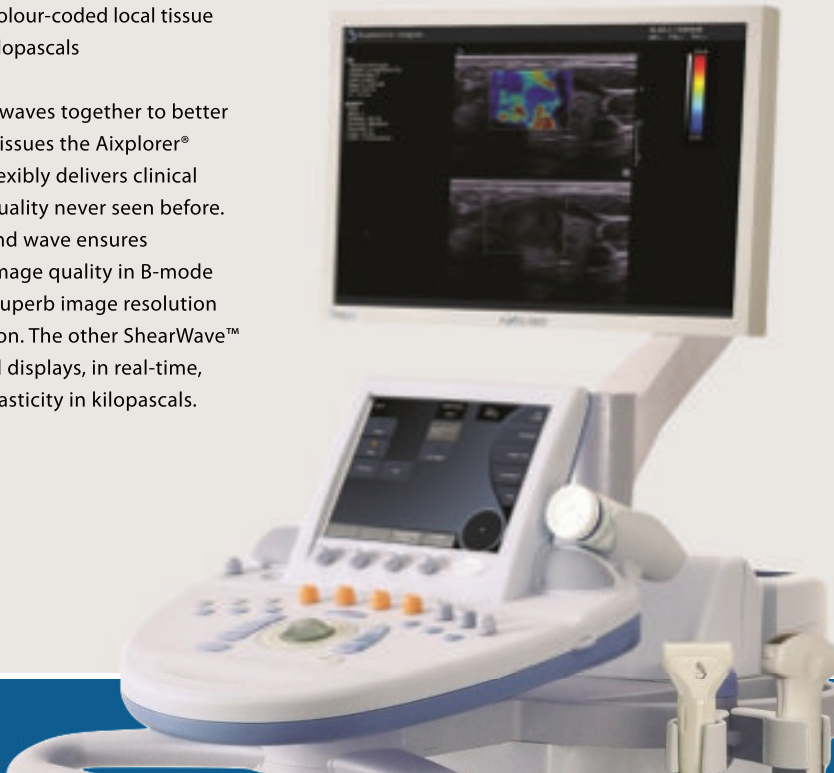
One ultrasound wave to ensure impeccable image quality
One shear wave to measure and display, in real-time, colour-coded local tissue elasticity in kilopascals

Bringing two waves together to better characterize tissues the Aixplorer® rapidly and flexibly delivers clinical images of a quality never seen before. One ultrasound wave ensures impeccable image quality in B-mode and ensures superb image resolution and delineation. The other ShearWave™ measures and displays, in real-time, local tissue elasticity in kilopascals.

With MultiWave Technology, Aixplorer relies on the simultaneous use of both ultrasound waves and shear waves to better characterize and quantify tissue stiffness. Shear wave velocity is directly related to the quantifiable measurement of tissue elasticity. Aixplorer can generate, capture and quantify the velocity of a shear wave by acquiring data up to 200 times faster than conventional ultrasound technology. Aixplorer produces a quantitative colour-coded map displaying local tissue elasticity for a large image region - in real-time. An easy-to-read colour scale in kilopascals indicates tissue elasticity.

Aixplorer relies on a revolutionary and patented software platform that acquires information at up to 20,000 images per second providing the greatest advances in image quality, leaving behind ultrasound insufficiencies, but also, the speed and capabilities for continuous innovation.

www.supersonicimagine.com



Hepatitis C Trust's Take Home Message

Jane Cox, Policy and Public Affairs Advisor/HCV Action Manager of the Hepatitis C Trust, gave the Treatment Strategies' team a brief explanation of the projects and services this UK national charity provides. In an interview with Treatment Strategies TV, Jane Cox goes on to share with us the Trust's take home message for the visitors of the International Liver Congress.

A UK national charity for hepatitis C, the Hepatitis C Trust was founded, led and run by people with experiences of hepatitis C. With the mission to reverse the rapidly increasing death toll caused by hepatitis C, the Hepatitis C Trust wants to reduce this preventable and treatable disease until it is eradicated from the UK.

The trust is involved in a range of projects and services to provide information, support for patients, raise awareness and increase research in this area.

Historically, hepatitis C has been neglected, partly because there has been no concerted patient voice. People with hepatitis C have typically kept quiet because it is infectious and because it has been wrongly stigmatized as 'a drug users' disease'. The Trust is committed to changing this, by:

- Raising public awareness that this is a virus that can be contracted in many ways

- Ending discrimination against people living with hepatitis C

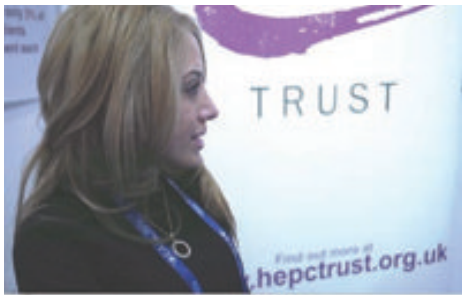
- Creating an active community of patients willing to stand up and be heard

- Providing information, support and representation for people with hepatitis C

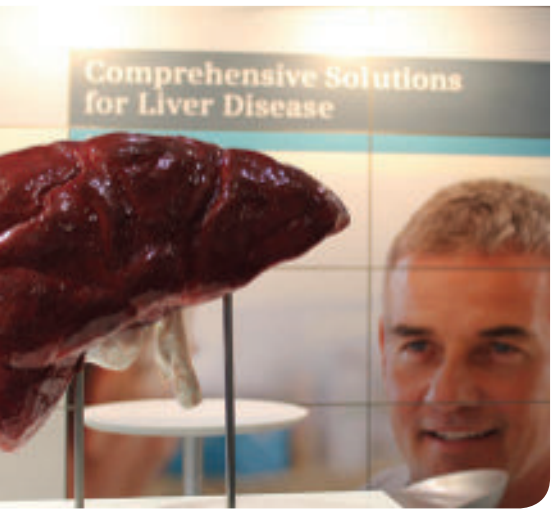
The Trust's mission is to reverse the rapidly increasing death toll caused by hepatitis C until no-one dies from this preventable and treatable disease and, ultimately, it is all but eradicated.

Jane went on to explain that it is a very exciting time for hepatitis C patients with many new treatments being developed with few side affects and good cure rates, which will help to eliminate hepatitis C in the future.

Please visit - **www.hepctrust.org.uk**



A New Standard in Liver Fibrosis Assessment



Siemens Healthcare is one of the world's largest suppliers of medical imaging, laboratory diagnostics and medical information technology.

With the Enhanced Liver Fibrosis (ELF™) test Siemens Healthcare hope to set a new standard in Liver Fibrosis Assessment. Liver fibrosis is the scarring process that represents the liver's response to injury or disease. All chronic liver disease (CLD) can lead to liver fibrosis and eventually cirrhosis, liver cancer, and death.

The major causes of liver fibrosis are:
- Viral hepatitis B and C infections
- Fatty liver disease (non-alcoholic fatty liver disease [NAFLD]) associated with obesity
- Alcohol abuse

Over 900 million individuals are at risk of chronic liver disease worldwide.

In response to chronic liver injury, stellate cells in the sinusoidal space are activated and deposit a collagen matrix (fibrosis). Over time, the fibrosis may become severe, leading to cirrhosis that may require a liver transplant or result in death.

At present in order to assess liver fibrosis, patients need to undergo an invasive liver biopsy. But due to there are many problems with obtaining and interpreting liver biopsies that have driven the search for additional methods to assess the severity of liver fibrosis.

A minimally invasive, standardised, and highly automated test for liver fibrosis assessment is the Siemens Enhanced Liver Fibrosis (ELF™). The Serum direct biomarkers of liver fibrosis used in this test make also make it an attractive option. The ELF test complements existing diagnostic tools to help manage patients with chronic liver disease.

The European Association for the Study of the Liver (EASL) approves use of non-invasive methods instead of liver biopsy to assess the degree of liver fibrosis in hepatitis C patients (EASL "Clinical Practice Guidelines: Management of hepatitis C virus infection," Berlin 2011).

The ELF Test (not available for sale in the U.S.) is a simple, standardised, routine blood test that allows you to assess the severity of liver fibrosis and is more convenient to request compared to other tests.

The ELF test can be used as an aid in the diagnosis and assessment of the severity of liver fibrosis in patients with signs and symptoms of chronic liver disease.

For more information please visit:
www.healthcare.siemens.co.uk

Non-Invasive Diagnosis and Monitoring of Liver Conditions

The Treatment Strategies' team met with Dr Banerjee from Perspectum Diagnostics, who provided a brief introduction on who Perspectum Diagnostics are and a detailed explanation of the LiverMultiScan.

Perspectum Diagnostics was founded by physicians, scientists, and engineers to exploit their patented technology and know-how to develop solutions for major unmet needs in diagnostic medicine in multiple internal organs. In the first instance Perspectum Diagnostics focuses on the detection and the accurate, quantitative measurement of liver, gallbladder and pancreatic disease, including precancerous and cancerous states in these organs. Currently there are no non-invasive quantitative and accurate analysis tools to diagnose them or monitor response to treatment.

Perspectum's initial focus is on liver disease, which already affects over 10% of the UK population and 15% of the US population. A fundamental challenge is that liver disease generally does not cause symptoms until tissue damage is severe and irreversible: for this reason it has been called 'the silent killer'. To date, the only accurate way to diagnose liver disease is with a needle biopsy. This is costly, often painful and has a significant risk of bleeding and a 0.1% risk of death. It is also often inaccurate due to sampling errors and many doctors and patients avoid liver biopsies because the risk seems to outweigh the benefits.

Perspectum Diagnostics use magnetic resonance imaging (MRI) to measure fat, iron and fibrosis in the liver. MRI is safe and does not use any radiation whilst examining the whole live in a single scan.

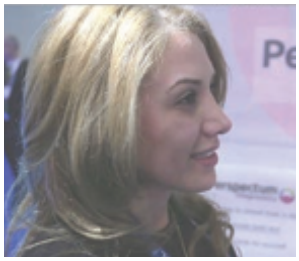
With the LiverMultiScan, Perspectum offers a non-invasive image analysis technology that provides an accurate assessment that can replace many liver biopsies by providing detailed information on tissue characteristics as well as architectural changes, thereby enabling a much more rapid and accurate diagnosis of liver disease than is possible with current blood and ultrasound tests.

Iron scoring may be assessed from a T2*-mapping image. MR spectroscopy and other sequences may be used to quantify fat content. Finally, a T1 mapping method that accounts for variations in intrahepatic composition can support quantification of fibrosis. Moreover, MRI provides high quality detail on the liver architecture, size and the presence of features such as dilated bile ducts or masses including liver tumours.

Perspectum's initial software product LiverMultiScan is expected to transform diagnostic hepatology by providing objective, quantitative and accurate information about patients' livers in one 20-minute visit, with no need for intravenous contrast.

The LiverMultiScan test is being evaluated in collaboration with clinicians and scientists at the University of Edinburgh, the NHR Birmingham Liver Biomedical Research Unit, and Perspectum Diagnostics. The trial is supported by a grant from the Technology Strategy Board and will run for two years.

For more information visit: **www.perspectum-diagnostics.com**



Indications Budenofalk® Preparations

Information was provided on the extensive variety of the indications Budenofalk® preparations have, by Dr. Falk Pharma GmbH at the International liver Congress in London.

Offering high and targeted efficacy, together with good tolerability, Budenofalk® preparations are a recognized treatment option in patients with inflammatory bowel diseases such as Crohn's disease, distal ulcerative colitis and collagenous colitis, as well as autoimmune hepatitis. Whilst other new indications for the locally acting steroid are currently being explored.

Budesonide, the active agent in Budenofalk® is a highly potent glucocorticoid that works differently to conventional systemic corticosteroids. The gastro-resistant formulations allow the pH release to be effective in inflamed areas of the gut and liver. Approximately 90% of budesonide then undergoes first pass metabolism in the liver before it enters the systemic circulation. This targeted efficacy in the gut and liver diseases significantly reduces the risk of systemic side effects.

Therefore when compared with conventional steroids, the action of budesonide remains confined to those bowel segments most commonly affected by inflammation in patients with inflammatory bowel diseases.

For more information please visit:
www.falkfoundation.org

Molecular Based INNO-LiPA™ Strip Assays for HBV

As pioneers within IVD testing solutions for HBV, our company has been present at the annual ILC/EASL meeting for many years, formerly under the name Innogenetics and for the first time this year as Fujirebio Europe.

At this year's ILC/EASL meeting Fujirebio focused on their well-established product portfolio of 3 molecular-based INNO-LiPA™strip assays for HBV: genotyping, drug resistance mutation testing and HBV precore & basal core promoter mutations.

Alongside with these now classical products Fujirebio also introduced their serological assays for the automated chemiluminescent LUMIPULSE® G1200 platform . The first range of HBV-assays on this platform will soon become commercially available on the European market. Among the HBV-assays in our portfolio is a novel marker called HBcrAg, Hepatitis B core related antigen. This promising new marker is currently being studied in several reference centers in Europe including The Hannover Medical School (MHH) in Hannover, Germany. This group presented initial HBcrAg data during the ILC/EASL meeting in the poster titled "Hepatitis B core-related antigen (HBcrAg) levels in the natural history of hepatitis B infection in a large European cohort.

The LUMIPULSE G platform also offers markers for the diagnosis and monitoring of hepatocellular carcinoma (HCC). In addition to AFP (á-fetoprotein) - which is part of current clinical practice - a marker called PIVKA-II (Protein Induced Vitamin K Absence or Antagonist) is now available on the platform supplementing AFP, especially in the detection of early HCC.

Please visit **www.fujirebio-europe.com** for more information.

Adsorption and Dialysis – An Effective Contribution to Liver Regeneration

The liver fulfils vital functions within the body including the production of proteins, the conversion and storage of metabolic products and – together with the kidneys – the detoxification of the blood. Acute or chronic liver failure can be caused by hepatitis, excessive alcohol consumption, poisoning, cancer or other reasons. A liver transplant is the only effective therapy for severe liver failure to date. However, in the past few years, scientists have developed blood-cleaning procedures that allow a patient to survive for at least several days or even weeks to bridge the time until the organ has recovered, or a donor organ has been found.

Liver dialysis is a detoxification treatment for liver failure and like a biological artificial liver device; it is a form of artificial extracorporeal liver support.

Liver dialysis is still in its infancy, unlike kidney dialysis, it cannot support a patient for an extended period of time. At present it is only considered to be a bridge to transplantation or liver regeneration. A critical issue in liver failure is the accumulation of albumin bound toxins in the liver. Hemodialysis is used for renal failure and primarily removes water-soluble toxins, however it does not remove toxins bound to albumin that accumulate in liver failure.

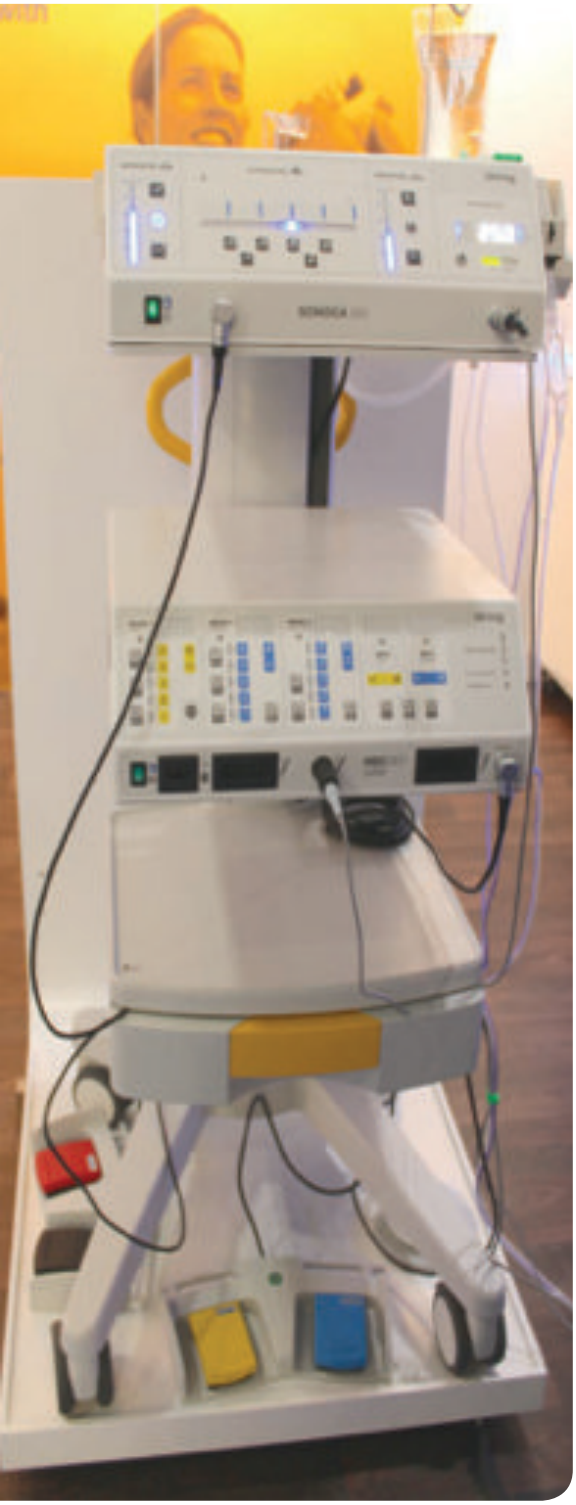
The development of artificial filtration and adsorption devices has enabled the removal of lipophilic, albumin-bound substances such as bilirubin, bile acids, metabolites of aromatic amino acids, medium-chain fatty acids and cytokines.

The Prometheus system – developed by Fresenius scientists in cooperation with the Danube University Krems is a new device based on the combination of albumin adsorption with high-flux hemodialysis after selective filtration of the albumin fraction through a specific polysulfon filter.

In contrast to dialysis used in kidney failure, liver failure also requires the removal of toxins that are bound to albumin, a transporting protein in the blood. Thus, the Prometheus system combines a typical dialysis procedure with an adsorber treatment. At first, the Prometheus machine pumps the blood through a newly developed filter (AlbuFlow) that retains blood cells and large protein molecules. The blood liquid, or plasma, along with albumin and smaller protein molecules is then fed through two adsorbers that separate toxins from the albumin and bind them. Following adsorption, the blood plasma with the detoxified albumin is joined with the blood cells retained by the AlbuFlow filter. Finally, the blood is dialyzed to remove the remaining water-soluble toxins, and the filtered blood is then reintroduced into the patient.

For more information visit: **www.fresenius.com**

Liver Surgery with Ultrasonic Technology



Söring, one of the pioneers in ultrasonic technology, has been providing the international market with innovative products for ultrasonic and high-frequency surgery for more than 25 years. At this years ILC™ they had on display their wide range of ultrasonic technology.

Gentle operating with ultrasonic technology is one of the most important developments in surgery.

The benefits are scientifically evident: The use of ultrasonic instruments leads to even better results with less loss of blood. In particular, the ultrasonic instruments by Söring not only minimise blood loss, but also prevent the formation of spray and smoke. They allow extremely precise preparation and coagulation without current flowing through the patient.

The major advantage of ultrasonic dissection lies in selective parenchyma transection. The ergonomic ultrasonic instruments by Söring are designed so that high selectivity of the tissue is achieved along with a high fragmentation rate. In addition, the precision instruments by Söring use both the cavitation as well as mechanical effect.

The liver parenchyma is reliably fragmented while the solid tissue structures of vessels and bile ducts remain unmarked. This significantly reduces the operative risk, even with special challenges such as a cirrhotic liver.

The selective application of ultrasound allows the surgeon to cut precisely, gently and with little haemorrhaging while preserving all the healthy structures.

A large spectrum of ergonomic handpieces and different ultrasound generators is continually being developed further for the most diverse of indications and allows extremely precise working – from delicate dissection to large-scale tissue removal.

With a wide range of technologies they produce, Söring is able to support surgeons throughout the entire operational procedure.

Further information available at:
www.soering.com

Non-Invasive Liver Stiffness Measurement Device

FibroScan®, a device used to measures stiffness and ultrasound attenuation (CAPTM), with a range of probes suitable for use on adults and children was showcased by Echosense. FibroScan® also allows for the management of patient data. It covers a broad range of applications, from initial diagnosis to predicting the complications of cirrhosis, and monitoring.

Examination with FibroScan®, is a non-invasive investigation which is also called transient elastography. The examination itself includes 10 consecutive measurements made at the same location. The results are provided immediately, and show the condition of the liver and by providing physicians with a tool to diagnose and monitor disease evolution in conjunction with treatment factors.

Exam results help to anticipate various complications, as well as to monitor and assess the damage caused by conditions such as cirrhosis. The FibroScan® examination is painless, quick and easy. During measurement, you feel a slight vibration on the skin at the tip of the probe.

Vibration-Controlled Transient Elastography (VCTETM) has provided an entirely new type of diagnostic tool. Many elastography machines are now available, giving physicians a range of new diagnostic equipment. All of these techniques can measure liver stiffness, but they do not all provide the same performance characteristics.

FibroScan®, based on the VCTETM technique, is the first medical device dedicated to the measurement of stiffness and CAPTM in the liver. Liver stiffness has been clinically validated via many studies carried out on thousands of patients and involving several different liver disorders. The measurement of liver stiffness by FibroScan® is painless for the patient, non-invasive, fast, and repeatable, and is a standardised procedure. It is also the first device that uses shear waves (see the inset on shear waves below). Shear waves have an amazing property in an elastic medium: their speed depends on the stiffness of the medium through which they are travelling. Inside the FibroScan®, an ultrasound sensor mounted on a vibrating system generates a low-frequency seismic wave (50 Hertz) between the ribs, on the surface of the skin.

Unlike radiation pressure techniques, which do not control the frequency of the shear waves, VCTETM can obtain perfectly repeatable measurements.
The speed of propagation of the waves is calculated using an ultra-fast ultrasound acquisition system, to measure movements in the liver. The speed of the shear waves depends on the hardness of the organ they are travelling through. The measurement obtained quantifies the hardness of the liver: the harder, and therefore the more fibrous the liver, the faster the wave propagation.

The result is not operator-dependent, and can be repeated as many times as necessary, because FibroScan® delivers very little energy to the patient.

In 2013, more than 2,000 FibroScan® units were being used all over the world. We estimate that more than 1.5 million men, women and children benefit from a FibroScan® exam every year.

For more information visit: **www.echosens.com**

Results from SAPPHIRE-I and SAPPHIRE-II Studies in Chronic Hepatitis C Patients

Results of the Phase III SAPPHIRE-I and SAPPHIRE-II studies were presented at the 2014 International Liver Congress™. The study found that SVR(12) rates of 96 percent were achieved in both SAPPHIRE-I (new to therapy) and SAPPHIRE-II (treatment-experienced with pegylated interferon and ribavirin) in adult patients with genotype 1 chronic hepatitis C virus infection.

In the SAPPHIRE-I (N=631) and SAPPHIRE-II (N=394) placebo-controlled studies, adult, non-cirrhotic patients with chronic genotype 1 (GT1) hepatitis C virus (HCV) infection receiving the investigational AbbVie regimen with ribavirin (RBV) for 12 weeks achieved sustained virologic response rates 12 weeks post-treatment (SVR12) of 96.2 percent (n=455/473) and 96.3 percent (n=286/297), respectively.

SAPPHIRE-I is a global, multi-center, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of 12 weeks of treatment with AbbVie's regimen with RBV in non-cirrhotic, GT1a and GT1b HCV-infected adult patients new to therapy. In SAPPHIRE-I, high response rates were seen across patients with certain variable characteristics, including gender, race, body mass index, fibrosis stage and baseline HCV viral load, as some of these patients have historically had a reduced response to treatment.

SAPPHIRE-II is a global, multi-center, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of 12 weeks of treatment with AbbVie's regimen with RBV in non-cirrhotic, GT1a and GT1b HCV-infected, treatment-experienced adult patients who previously failed treatment with pegylated interferon and RBV. In SAPPHIRE-II, treatment-experienced sub-populations randomized to the AbbVie regimen with RBV were prior null responders (49.2 percent), prior relapsers (29.0 percent) and prior partial responders (21.9 percent) to pegylated interferon and RBV.

"These data provide further evidence that AbbVie's regimen can achieve high SVR12 rates across a range of GT1 patients with varying prior treatment experience and response," said Scott Brun, M.D., Vice President, Pharmaceutical Development, AbbVie.

Results from the Global HALLMARK-Dual study

Results from the global HALLMARK-Dual study were presented by Bristol-Myers Squibb at The International Liver Congress™.

This Phase III trial investigated the all-oral, interferon- and ribavirin-free regimen of daclatasvir (DCV) a NS5A inhibitor, and asunaprevir (ASV), a NS3 inhibitor, among genotype 1b hepatitis C virus (HCV) infected patients.

Results showed that the investigational all-oral Daclatasvir and Asunaprevir therapy achieved SVR12 rates of up to 90% among broad range of genotype 1b hepatitis C patients.

In the study the DCV+ASV regimen was generally well tolerated. These data will be presented this week at the 49th annual meeting of the European Association for the Study of the Liver (EASL) The International Liver CongressTM in London, April 9-13.

This Phase III multinational clinical trial involved 116 sites in 18 countries, including countries that have a high prevalence of genotype 1b such as Korea and Taiwan. In the study, treatment-naïve patients (n=205) received DCV 60 mg once daily plus ASV 100 mg twice daily for 12 weeks, and 102 patients received matching placebo for 12 weeks. The DCV+ASV treatment-naïve group continued treatment through week 24; placebo recipients entered another DCV+ASV study. The peginterferon/ribavirin-ineligible/intolerant (n=235) and non-responder patients (n=205) received the same doses of DCV and ASV for 24 weeks. The primary endpoint was the percentage of patients with a sustained virologic response at 12 weeks after the end of treatment (SVR12). The results of the HALLMARK-Dual study include data among genotype 1b cirrhotic and non-cirrhotic, treatment-naïve, non-responder, and peginterferon/ribavirin ineligible and intolerant patients.

This study reinforces the potential of daclatasvir-based regimens to treat HCV patients with high unmet needs.
Please visit: **www.bms.com**

Data from Cohort 2 of the Phase 2 COSMOS Study

Positive new data from the clinical development programme of simeprevir was presented at The International Liver Congress™ 2014 of the European Association for the Study of the Liver (EASL) in London.

Simeprevir is an NS3/4A protease inhibitor jointly developed by Janssen R&D Ireland and Medivir AB and indicated for the treatment of chronic hepatitis C infection in combination with pegylated interferon and ribavirin in genotype 1 HCV infected patients with compensated liver disease, including cirrhosis.

This data included final data from cohort 2 of the Phase 2 COSMOS study of the protease inhibitor simeprevir administered once daily with Gilead's nucleotide inhibitor sofosbuvir, with and without ribavirin (RBV), in adult patients chronically infected with genotype 1 hepatitis C virus (HCV).

"The efficacy seen with the combination of simeprevir and sofosbuvir is very promising, especially considering the inclusion of patients with more advanced liver fibrosis in Cohort 2," said Dr. Eric Lawitz, M.D., simeprevir clinical trial investigator, CEO at The Texas Liver Institute and Alamo Medical Research and Clinical Professor of Medicine at University of Texas Health Science Center.

"I look forward to seeing the combination of simeprevir and sofosbuvir further evaluated in the recently initiated Phase 3 OPTIMIST trial."

"The data presented at EASL further reinforce the benefit of simeprevir-based treatment across diverse patient populations, including European patients," said Gaston Picchio, Hepatitis Disease Area Leader, Janssen Research & Development. "Following the recent positive opinion for simeprevir from the Committee for Medicinal Products for Human Use in the European Union, we look forward to bringing this regimen to patients in Europe in the near future."

Please visit: **www.janssenrnd.com**

All Oral Treatment Regimens for HCV

MSD a global healthcare leader has been at the forefront of the response to the HCV epidemic for over 25 years. At the International Liver Congress™, MSD presented additional data from the on-going C-WORTHy study. This is a two-part, parallel-group, randomized clinical trial evaluating a range of subpopulations of patients with hepatitis C virus (HCV) Genotype 1 infection (GT1) infection. A multi-arm Phase 2 clinical trial this study evaluated the efficacy and safety of a once-daily, all-oral regimen combining MK-5172, an investigational HCV NS3/4A protease inhibitor, and MK-8742, an investigational HCV NS5A replication complex inhibitor, among patients with chronic HCV GT1.

In an interim analysis of treatment-naïve, non-cirrhotic patients were observed for different treatment durations of MK-5172 plus MK-8742 with or without RBV. A total of 471 patients with HCV GT1 RNA le were enrolled in C-WORTHy across 16 arms.

A sustained viral response1 (SVR) was observed in 98 per cent (42/43) of patients administered MK-5172/MK-8742 alone and 94 per cent (75/80) in those administered MK-5172/MK-8742 plus RBV.

The interim results presented were from treatment-naïve, non-cirrhotic patients who received one of 3 regimens: A) MK-5172/MK-8742 + RBV for 8 weeks (N=30), B) MK-5172/MK-8742 + RBV for 12 weeks (N=85), and C) MK-5172/MK-8742 (without RBV) for 12 weeks (N=44).

Among the five patients who relapsed in the eight-week regimen arm of PN035B, two patients had very low MK-5172 and MK-8742 levels during the course of therapy. The relationship between this finding and the relapse is currently being investigated.

The most common adverse events recorded in the RBV and RBV-free treatment groups, respectively, were fatigue (32%, 23%), headache (20%, 33%), nausea (21%, 16%), diarrhea (13%, 9%) and insomnia (13%, 7%). There were no early discontinuations due to drug-related adverse events and no clinically significant abnormalities detected in routine laboratory analysis of hematologic markers.

Based on the results of the Phase 2 clinical program, Merck has initiated Phase 3 clinical trials for MK-5172/MK-8742, called C-EDGE to evaluate the safety and efficacy of MK-5172/MK-8742 with and without ribavirin.

For more information please visit: **www.merck.com**

Hepatic Encephalopathy Contributes to the Burden of Liver Disease

Norgine, a leading independent European specialty pharmaceutical company, was showcasing XIFAXAN® 550 (rifaximin-α), a cost-effective treatment option for the reduction of recurrent hepatic encephalopathy.

Norgine presented data during their symposium 'The Hidden World of HE' which showed the impact of hepatic encephalopathy (HE) on liver disease patients and healthcare systems. Patients who develop HE are at a higher risk of dying compared with similar liver disease patients without HE.

Further analysis demonstrates that liver disease patients with HE are admitted to hospital more frequently and collectively representing a substantial increased use of healthcare resources.

Currently there is no cure for HE other than a liver transplant, however, XIFAXAN® 550 (rifaximin-α) has shown to reduce the risk of further HE episodes.

XIFAXAN® 550 is a broad spectrum antibiotic that targets commensal gut bacteria, acting on Gram-negative and Gram-positive aerobes and anaerobes, reducing the excess ammonia produced by the gut bacteria of patients with cirrhosis.

"Hepatic encephalopathy is a serious but largely un-recognised condition that must be considered as part of the overall burden of liver disease." commented study investigator Dr Mark Hudson, Consultant Hepatologist, Freeman Hospital, UK.

Dr Hudson added: "These new data demonstrate that hepatic encephalopathy both significantly increases mortality risk in patients with chronic liver disease and places a substantial additional burden on already-stretched healthcare systems in both primary and secondary care. XIFAXAN® 550 is an important new medicine in the management of hepatic encephalopathy, and the cost-effectiveness data presented today support its benefits in terms of potential cost savings versus current practice."

For more information please visit: **www.norgine.com**

Poster Synopsis

Gene Expression Analysis Identifies Immune Signaling and Complement Pathways in IgG4-related Disease

Emma L Culver,[1,2] Emanuele Marchi,[1] Tamsin Cargill,[1] Wouter Smit,[1,3] Mateusz Makuch,[4] Narayan Ramamurthy,[1] Theo Rispens,[4] Paul Klenerman[1] and **Eleanor Barnes[1,2]**

1. Peter Medawar Building, Nuffield Department Medicine, Oxford University; 2. Translational Gastroenterology Unit, John Radcliffe Hospital, Oxford; 3. Academic Medical Centre, Amsterdam, The Netherlands; 4. Sanquin Blood Supply, Amsterdam, The Netherlands.

Introduction

IgG4-related disease is a corticosteroid-responsive systemic fibro-inflammatory condition of unknown aetiology. Diagnosis relies on careful interpretation of a combination of histopathological appearances, radiological features and serological abnormalities, in an appropriate clinical scenario..To identify the potential mediators, effector molecules and biological pathways implicated in disease pathogenesis we used a systems biology approach.

Methods

Whole blood genome oligo-microarray was performed in 8 treatment-naïve patients with active IgG4-RD (IgG4 related cholangitis and autoimmune pancreatitis; of whom 4 had additional systemic manifestations) and 8 healthy age and sex-matched controls. Gene expression profiles were interpreted using Ingenuity Pathway Analysis (IPA) software and the Database for Annotation, Visualisation and Integrated Discovery (DAVID) web-based program. Gene Set Enrichment Analysis was used to assess co-expression of gene sets with shared biological function or regulation. Gene Set Enrichment plots and Heat Maps were created. Gene expression was confirmed at the RNA level by Taqman RT-PCR and at the protein level by ELISA, cytokine array, and flow cytometry. A deconvolution algorithm was applied to infer the contribution of each cell type to the general expression profile, using an R package for cell-type specific differential expression of microarray experiments in heterogeneous samples.

Results

213 putative candidate genes were identified: 161 up-regulated (fold>2, p<0.01) and 52 down-regulated (fold<-2, p<0.01), shown in figure 1. Pathway analysis revealed 16 immunologically functional genes and 2 pathways implicated in disease pathogenesis, shown in figure 2. Genes encoding cytokines (IL5 and IL21), the chemokine ligands (CCL23 and CCL25), and the classical complement and TGF beta pathways were shown to be active in the disease cohort compared to healthy controls, which was confirmed by RT-PCR. Gene set enrichment analysis confirmed enriched gene sets with shared biological function in IgG4-RD patients compared with controls (figure 3). Genetic markers were identified which were specifically enriched in the group with systemic manifestations, compared to single organ disease. Deconvolution indicated the monocytic lineage was important in differential gene expression.

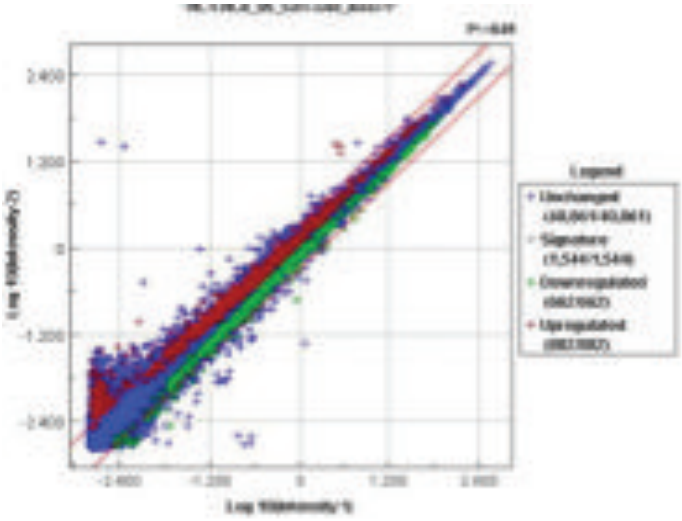


Figure 1. Double Log Scatter Plot of signal intensities in 8 treatment-naïve IgG4-RD patients compared with 8 matched healthy controls. The signal intensities of each feature represented by a dot are shown in the double log scale. Red diagonal lines define the area of 2 fold different signal intensities.

Conclusion

Gene expression analysis in IgG4-RD implicates antibody inducing cytokines, hepatic/biliary chemokines and the complement pathway as putative immune drivers of disease, providing novel targets for disease stratification and therapeutics.

Funding: EL Culver receives funding from a Wellcome Trust Research Training Fellowship into the natural history and pathogenesis of IgG4-RD [095160/Z10/Z].

Emma Culver graduated from Edinburgh University with an intercalated BSc in Neuroscience in 2001, MBChB in 2004 and E. Culver attained her MRCP in 2007. She embarked on Speciality Registrar training in Oxford, dual accrediting in Gastroenterology and General Medicine. She is now in the final year of her DPhil in Clinical Medicine investigating the natural history and pathogenesis of IgG4-RD, an NIHR portfolio study funded by the Wellcome Trust.

Poster Synopsis

Figure 2. Full table of genes and their immunological function as identified by pathway analysis using IPA and DAVID.

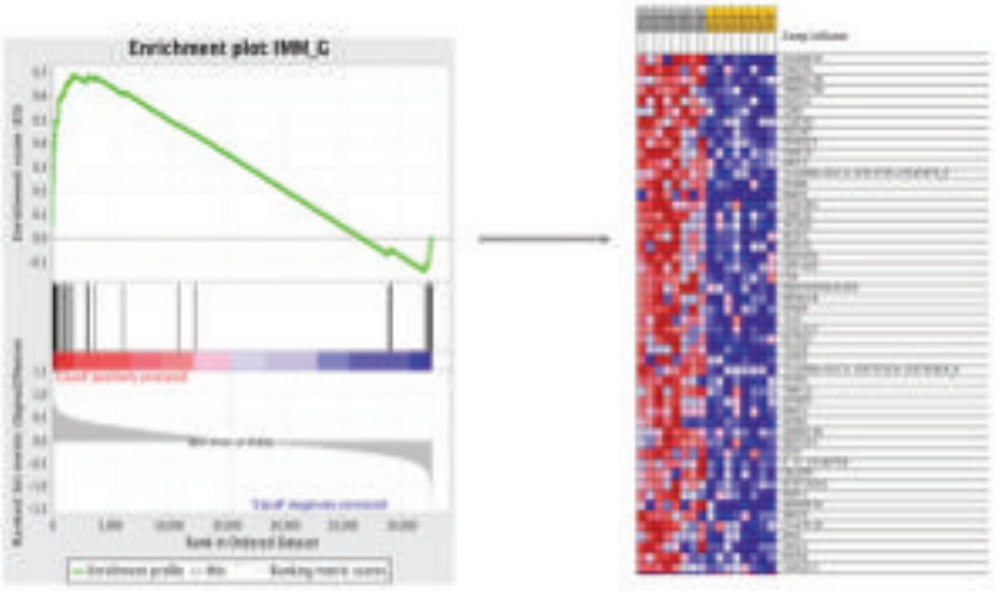


Figure 3. Gene Set Enrichment plot and heat map demonstrates enriched genes (p<0.01) with shared biological function in IgG4-RD compared to healthy controls.

Poster Synopsis

Effectiveness of Different IFN-α Regimens in Antiviral Treatment of HDV-Infection

K.I.Yesmembetov,[1,2] D.T.Abdurakhmanov[1] and **N.A.Mukhin[1]**

1. I.M.Sechenov First Moscow State Medical University, Moscow, Russia; 2. Liver Centre, City Hospital #1, Astana, Kazakhstan

Background and Aims

Hepatitis delta virus (HDV) is a defective RNA virus, which requires hepatitis B virus (HBV) for transmission and replication, causing the most severe type of viral hepatitis infection in humans[1].

Several treatment options, including nucleos(t)ide agents (clevudine[3], lamivudin[4], famciclovir5, ribavirin[6], adefovir[7]) and IFN-α containing regimens have been tried so far during last 3 decades, best results being achieved by current standart-of-care antiviral agent - PegIFN-α2, effective in only about 25% of patients[2].

We aimed to assess the efficacy of different IFN-α regimens in patients with HDV-infection.

Methods

102 patients with serological markers of HDV-infection (anti-D and HDV RNA positive), followed-up from Jan 2002 to Jan 2011 in the hepatology department of the E.M.Tareyev clinic, I.M.Sechenov First Moscow State Medical University, were included in the study.

Results

Out of 102 patients (mean age 39 years, male 57.8%) with HDV-infection only 49 (48%) were eligible for treatment with IFN-α. 40 (39,2%) patients (male 26, mean age 35,8 years, mean BMI 23,7), which have completed at least 26 weeks of treatment with IFN-α were included in the analysis. 7 (17,5%) out of 40 patients were treated with human leukocyte Interferon-α (HuIFN-α-Le), 1,5-6 MU daily for 24-48 weeks. Sustained biological response (SBR, normal ALT 24 weeks after treatment completion) was achieved in 3 (42,9%) patients, but none of them demonstrated sustained virological response (SVR, undetectable HDV RNA 24 weeks after treatment completion). 16 patients were treated with either IFN-α2a or IFN-α2b 3MU thrice weekly or 5MU daily for 24-72 weeks. 31,3% of them demonstrated SBR, but only 25% achieved SVR. 20 patients were treated with PEG-IFN-α2a/PEG-IFN-α2b for 24-108 weeks. 40% of them achieved SBR, while 25% achieved SVR.

Stage of the Disease	Mean age,years	Number of Treated Patients	SVR rate %0%
Acute Hepatitis d	48	1	0%
Chronic Hepatitis D	30.1	19	47.3%
Compensated HDV-Cirrhosis	39.7	18	57.6%
Decompensated HDV-Cirrhosis	49.5	2	0%

Table 1.

Thus, majority of patients, achieved SVR were young and had chronic hepatitis (table1), which is consistent with the results of similar studies[8].

Conclusions

PEG-IFN-α2 remains the most effective antiviral agent for HDV-infection, providing SBR and SVR in 40% and 25% of the patients, respectively. Rate of antiviral response was dependant on disease stage with the most prominent SVR rates achieved in chronic hepatitis patients.

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Kakharman Yesmembetov is a hepatologist and the head of liver centre at the City Hospital #1, Astana, Kazakhstan. He has completed his medical post-graduate course and defended PhD thesis at the I.M.Sechenov First Moscow State Medical University, Moscow, Russia. Dr. Yesmembetov's current research interests cover areas of pre- and post-liver transplantation management of patients.

Poster Synopsis

Syndecan-1 as a Cofactor of CD81 in Hepatitis C Virus Infection of Hepatocytes

Boyan Grigorov,[*1] Alice Gentil-dit-Maurin,[*2] Mihayl Varbanov,[*2] Julie Blaising,[1] Maud Michelet,[1] Fabien Zoulim,[1] Florence Ruggiero[3] and **Eve-Isabelle Pécheur[1]**

*equal contribution; 1. CRCL, Inserm U1052, CNRS 5286, University of Lyon, Lyon, France; 2, IBCP, UMR CNRS 5086, University of Lyon, Lyon, France; 3, IGFL, UMR 5242 CNRS - ENS Lyon, Lyon, France

Hepatitis C is a major health concern, with 170 million people infected worldwide. It is a chronic infectious disease caused by the hepatitis C virus (HCV), leading to liver cirrhosis and hepatocellular carcinoma (HCC) in severe cases.

HCV is a strictly human pathogen which brings about productive infection in hepatocytes. In the perspective of deciphering HCV hepatotropism, we aimed at investigating the role of the hepatic microenvironment (HME) in HCV entry.

It is achieved through the recognition of several receptor molecules, including the tetraspanin CD81, the cholesterol transporters SR-BI and NPC1L1, tight junction components claudin-1 and occludin, the EGF and the transferrin receptors.

Since these molecules are expressed in various cell types and tissues, we focused on heparan sulfate proteoglycans (HSPG) of the HME, which bear tissue specificity and transduce signals intracellularly, and studied the potential involvement of syndecan-1 (synd-1).

We found that synd-1 was exposed at the blood pole (basolateral membrane) of primary human hepatocytes, by which the virus reaches the cells, with minimal to no colocalisation with specific markers of the bile pole (apical membrane).

Specific strategies using antibodies to synd-1, or siRNAs silencing synd-1 or xylosyltransferase-1 (a key upstream enzyme in HSPG biosynthesis) drastically impaired HCV infection.

We observed a downregulation of synd-1 in HCV-infected hepatoma cells, similar to what was described for some HCV entry factors.

Synd-1 colocalised with virions at the cell membrane at early stages of entry, and later in endosomal structures inside the cell, positive for the small GTPase Rab5.

The recognition of synd-1 by HCV strictly required the presence of heparans sulfates on synd-1 ectodomain.

Proximity ligation assays revealed that synd-1 strongly colocalised with CD81 in uninfected primary or transformed hepatocytes, and both molecules could be co-immunoprecipitated.

At early stages of HCV infection (hours post-infection), synd-1 colocalised with virions and CD81 in endosomal compartments.

Since virion internalisation in these CD81-positive compartments was found related to productive infection, this suggests that synd-1 acts as a co-factor of CD81 for HCV infection.

At later stages of infection (days post-infection), this colocalisation vanished and concomitant disorganisation of cellular junctions was observed.

Collectively our data point to a role played by synd-1 in HCV infection, likely in conjunction with CD81. This peculiar combination might partly contribute to HCV hepatotropism.

Our ongoing investigations will help define which synd-1-dependent signalling cascade(s) is initiated at the onset of HCV infection.

Boyan Grigorov is currently Assistant Professor at the Faculty of Pharmacy of the University Lyon 1, and works in the group of E-I. Pécheur. After completing his Masters degree in Molecular Biology, obtained in Sofia, Bulgaria, he obtained his Ph.D at the Ecole Normale Supérieure of Lyon, France, working on HIV-1 assembly and cell-to-cell transmission. His expertise in virology was applied to HIV-1 drug discovery and to the optimisation of a vaccine against the measles virus, as an R&D scientist at Sanofi Pasteur. He is currently aiming at unraveling the potential interplay between hepatitis C-induced oxidative stress, alterations in the liver microenvironment and the early steps of hepatocarcinogenesis.

Eve-Isabelle Pécheur is a group leader at the Cancer Research Center of Lyon, France. She graduated from the Faculty of Pharmacy of the University of Rennes, France, in 1992. She obtained her Ph.D. in Pharmaceutical Sciences at the University of Paris XI in 1997, with the specialty of Biopharmacy and Galenic Pharmacy. She has been a post-doc fellow at the Medical School of the University of Groningen, The Netherlands. Her main research interests are the role of the hepatic microenvironment in the infection by the hepatitis B and C viruses and the discovery of novel antiviral strategies. She is the author of 50 publications, and of several research works presented in national and international symposia.

Poster Synopsis

Protective Effects of Silybin in an *in vitro* Model of Non-alcoholic Fatty Liver Disease

Veronica Marin,[1] **Claudio Tiribelli**[1] and **Natalia Rosso**[1]
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Background and Aim

Non-alcoholic fatty liver disease is a chronic disorder that affects about 15-40% of the world population,[1] especially in Western Countries. It is widely recognized as a component of the emerging obesity epidemic and it is defined as the hepatic manifestation of Metabolic Syndrome. The condition is asymptomatic, benign and completely reversible. NAFLD includes a disease spectrum ranging from simple steatosis to the inflammation development (Non-alcoholic Steatohepatitis), until more severe stages of fibrosis and cirrhosis.[2]
No specific therapy is available so far. Changes in the quality of life and diet habits are the most efficient choices, but patients compliance toward this approach is the main limitation.[3] An emerging alternative is based on the introduction of dietary supplement, mainly natural compounds, to create new formulations able to improve the pathological features of NAFLD. The aim of the present study is to assess the therapeutic properties of Silybin, which is the main active compound of Milk Thistle.[4]

Materials and Methods

All the experiments were carried out using an *in vitro* model developed by our group,[5] which reproduces the main features of this pathology. Hepatic cell line (HuH7), was exposed for 24 h to 1200µM of a mixture of FFA 1200µM (Oleic: Palmitic 2:1) to reproduced the events observed in steatohepatitis. In order to determine the therapeutic effects of the compound, cells were exposed at the same time to FFA and different concentrations of Silybin (1, 5, 7.5µM). Cell viability was assessed through MTT test. Through the flow cytometry analysis were assessed: presence of steatosis and apoptosis using Nile Red and Annexin V/ Propidium Iodide staining respectively, and study of the cell cycle. mRNA expression of inflammatory cytokines (IL8, TNF-α) was determined by RT-real Time PCR. Oxidative stress, in term of Reactive Oxygen Species (ROS) generation, was measured fluorometrically by quantification of DCF. Hepatic transaminases released in the culture medium, such as ALT and AST, were quantified by kinetic activity assay.

Results and Conclusions

1µM Silybin induced a significant ($p<0.05$) reduction in mRNA expression of IL-8 and TNF-α (1.30±0.30 and 1.76±0.4 fold, respectively) vs. FFA treated cells. On the contrary, no change in the intracellular fat accumulation was observed. Regarding ROS levels, only the highest dose of Silybin (7.5µM) reduced after 1 hour the oxidative stress induced by FFA (35± 17% $p<0.05$). In line with this data, t 7.5µM Silybin reduced the number of cells undergoing to apoptosis 10.6±1.2% ($p<0.01$) vs. FFA after 24h. Surprisingly, in absence of FFA Silybin exerted an acute inflammatory response, promoting the up-regulation of TNF-α (8.69± 0.39 fold, $p<0.02$) and IL8 (5.53±0.10 fold $p<0.001$) vs. vehicle treated cells. Consequently, an increased number of apoptotic cells (25±3% $p<0.01$) were observed. To better understand the Silybin double activity, we analyzed the cell cycle. The results showed that Silybin, when used alone, induced cell cycle alteration at all the concentrations. The co-treatment with FFA did not induce further changes.
All together, these data suggest that the powerful effect of Silybin is related with its anti-inflammatory and anti-oxidant properties. This finding is clinically promising since those are the two key player involved in the progression of this hepatic disorder. Further studies are necessary to confirm the potential benefits of Milk Thistle as dietary supplement in NAFLD treatment. In addition, the use of this compound in a non steatotic condition deserves further clarifications.

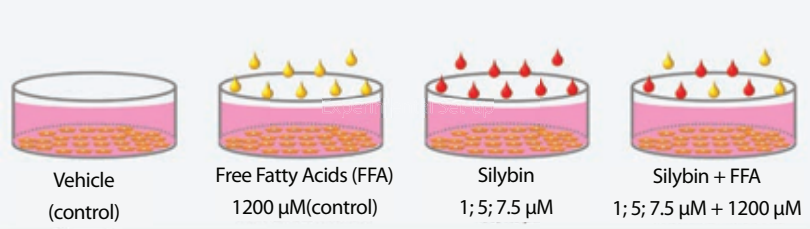

Figure 1: Schematic representation of the experimental set-up. Hepatic cells (HuH7) exposed to different treatments for 1 or 24 h.

Acknowledgements

This study was supported by MIUR (Art.13 D.LGS 297/99- Progetto Nutrizione e Salute).

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Veronica Marin graduated in Pharmaceutical Chemistry and Technology, University of Trieste. She is following her PhD program on Molecular Hepatology, at the School of Molecular Biomedicine, Italian Liver Foundation laboratories. Her research interests are focused on the study *in vitro/ in vivo* of the underlying mechanisms involved in NAFLD/ NASH, especially towards the pediatric population.

Poster Synopsis

Cost Effectiveness of Rifaximin-a in the Reduction of Recurrence of Overt Hepatic Encephalopathy

Chris D. Poole, Peter Conway,[2] Kam Nanuwa,[3] Bimpe Joseph,[2] Christian Bannister and **Craig J. Currie[1]**
1. School of Medicine, Cardiff University, UK; 2. Norgine Global Health Outcomes, Uxbridge, UK; 3. Norgine UK, Uxbridge, UK

Introduction

Hepatic encephalopathy (HE) is a serious neuropsychiatric condition associated with high morbidity and mortality. It is characterised by an underlying impairment in neurocognitive function which is termed minimal or covert HE. This can progress to a more severe state, in which patients experience episodes of neurological dysfunction, lasting from several hours up to several days or even weeks and some patients may end up in a coma. This is known as an overt state. Rifaximin-a is effective in reducing the recurrence of HE events and associated hospitalisations.[1]

Aims

The aim was to characterise the cost effectiveness of rifaximin-a in addition to standard care versus standard care alone in UK clinical practice. Standard care is defined as maintenance lactulose therapy.

Methods

- Design of analysis
  - A cost utility analysis was conducted using a Markov state transition model, depicting a simplified representation of the disease process, as validated by a number of clinical experts (Figure 1)
  - The model has Markov health states describing covert (C1) HE, overt (01) HE and death. Patients experiencing overt HE in the model either die or return to a second covert health state (C2) which assumes a higher risk of mortality but no decrease in impact on quality of life (QoL)
- Target population
  - Adult patients with chronic liver disease who have experienced at least one overt HE episode
  - The patient population reflects the patients characteristics of those observed in the pivotal study1 and the open label extension study (OLE)[2]
- Setting and study perspective
  - The payer perspective was that of the UK National Health Service. Both outpatient and inpatient treatment settings were considered
- Interventions being evaluated
  - Treatment with rifaximin-a + standard care (rifaximin-a group)
  - Placebo+ standard care (standard care group)
- Time horizon
  - An updated model evaluated a 5-year, 1 0- year and lifetime horizon (42 years= death of last patient)
- Annual discounting was applied at a rate of 3.5% for both costs and benefits
- The health outcome was the QALY, the standard unit of measurement for benefit required by the UK National Institute for Health and Care Excellence (NICE)
  - QALYs were determined by modeling transitions from covert to overt HE states and death, and applying utility weightings to each health state
- Required model inputs
  - Time to first observed HE event was determined from the pivotal trial
  - Time to all subsequent events was determined from an OLE study
  - Time to death was determined from the observed mortality rates in the OLE
- Measurement of QoL and quantification of health-related utility
  - QoL was measured during the pivotal study during the covert state only using the
    - Chronic Liver Disease Questionnaire (CLDQ) a disease specific QoL instrument)
    - Short Form 36 (SF-36) a generic QoL instrument
  - Assessments were not made during an overt HE breakthrough episode due to the patients altered mental and neuromotor status
  - Patients were excluded from the study if they experienced a breakthrough episode of HE
  - The average duration of disutility from an overt episode was estimated by a panel of clinical experts to be 11 days[3]
- Estimating resources and costs
  - Costs were based on data from published sources and were in UK pounds sterling at 2012 prices.
  - Drug acquisition costs were £289.95 and £9.09 for rifaximin-a and lactulose per model month (30. 4 days), respectively[4]
  - The total estimated cost of an HE-related admission was £1,040.77 per episode[5] for which:
    - The average length of stay in hospital was estimated by a panel of clinical experts to be 5 days[3]
    - The likelihood of an admission to hospital was 52.88%[1]
  - The cost of an outpatient consultation was £176.275
- Evaluation of uncertainty
  - The impact of uncertainty in the input parameters on the model was explored with one-way and probabilistic sensitivity analysis (PSA)

Results

- In this model- based analysis; treatment with rifaximin-a versus standard care would demonstrate:
  - Reduced progression to HE events
  - Improved patient survival through reduced risk of mortality due to reduction in HE episodes
  - Improved health-related utility during remission I covert states
  - Rifaximin-a appears to offer a cost-effective treatment option for reduction of recurrence of overt HE assuming a cost effectiveness threshold of £30,000 per QALY
- Modelled mortality was a close visual fit to that observed in the open-label extension study and also that observed in matched cohort drawn from the UK Clinical Practice Research Database (CPRD) database (Figure 2) [6]
  - The incremental cost effectiveness ratio (ICER) were £20,829, £19,207, and

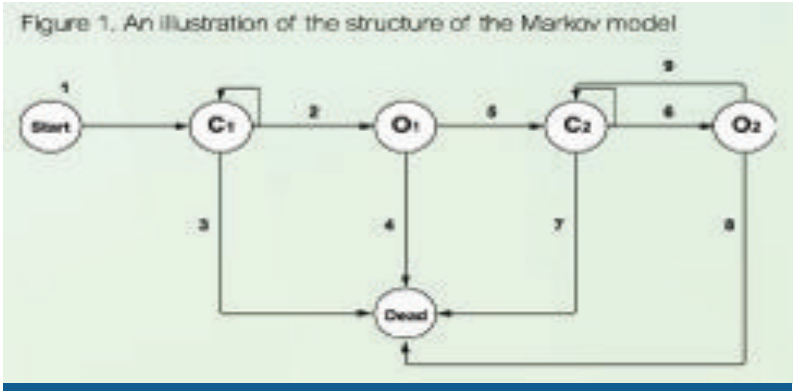

Figure 1. An illustration of the structure of the Markov model. Key - Patients enter the model in the remission state. Covert State (C1) to first-observed over episode (01). Covert State (C1) to death. First-observed overt episode (01) to death. Recovery from first-observed overt episode (02) to subsequent covert state (C2). Subsequent covert state (C2) to subsequent overt episode (02). Subsequent covert state (C2) to death. Subsequent overt episode (02) to death.

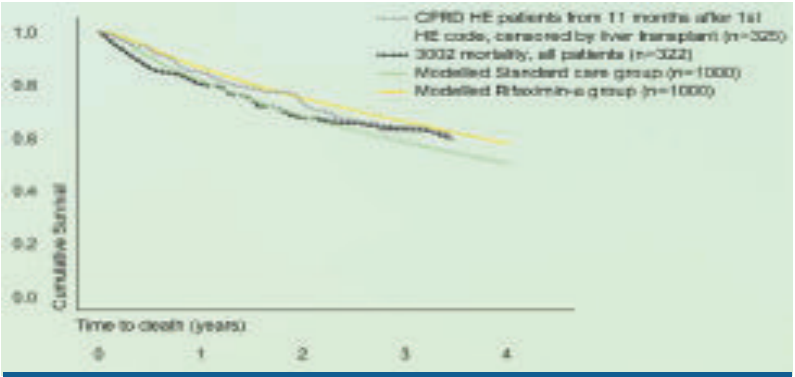

Figure 2. Comparison of modelled survival with observed survival in the open-label extension study and post-HE survival in a matched observed population drawn from the UK CPRD database.

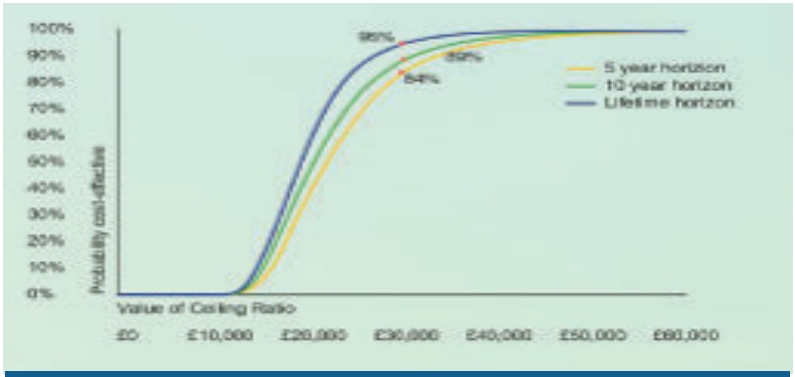

Figure 3. Cost Effectiveness Acceptability Curve (CEAC) for 5, 10 and lifetime horizon.

Time horizon (years)	Discounted costs – rifaximin-a group	Discounted costs – standard care group	ICER
5	£15,559	£4,574	£20,829
10	£22,358	£5,887	£19,207
42 (Life-time)	£28,874	£6,925	£17,681

Table 1. Incremental cost effectiveness ratios over 5, 10 and lifetime horizon.

£17,681 with a time horizon of 5 years, 10 years and lifetime, respectively (Table 1)

- Probabilistic sensitivity analysis (PSA)
  - In the 5-year, 1 0-year and lifetime horizons, rifaximin-a was found to have an 84%, 89% and 95% likelihood of being cost-effective at WTP of £30,000 per QALY, respectively (Figure 3). The probabilistic ICER was £21 ,000, £19,555 and £18,202

Conclusions

- This model was developed to reflect real world clinical practice, disease progression and related outcomes
- Under these conditions rifaximin is shown to be cost effective by reducing recurrence of overt HE episodes. This remains true under a broad range of clinically plausible scenarios

Limitations

There are limitations to this model since it was based on a combination of trial and epidemiological sources combined with opinions from clinical experts.

As there was no placebo data in the open label extension study, assumptions were validated using real world data derived from CPRD as well as clinical expert opinion. The PSA, however, demonstrated stability of the final estimated ICER.

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50th Annual EASL Congress
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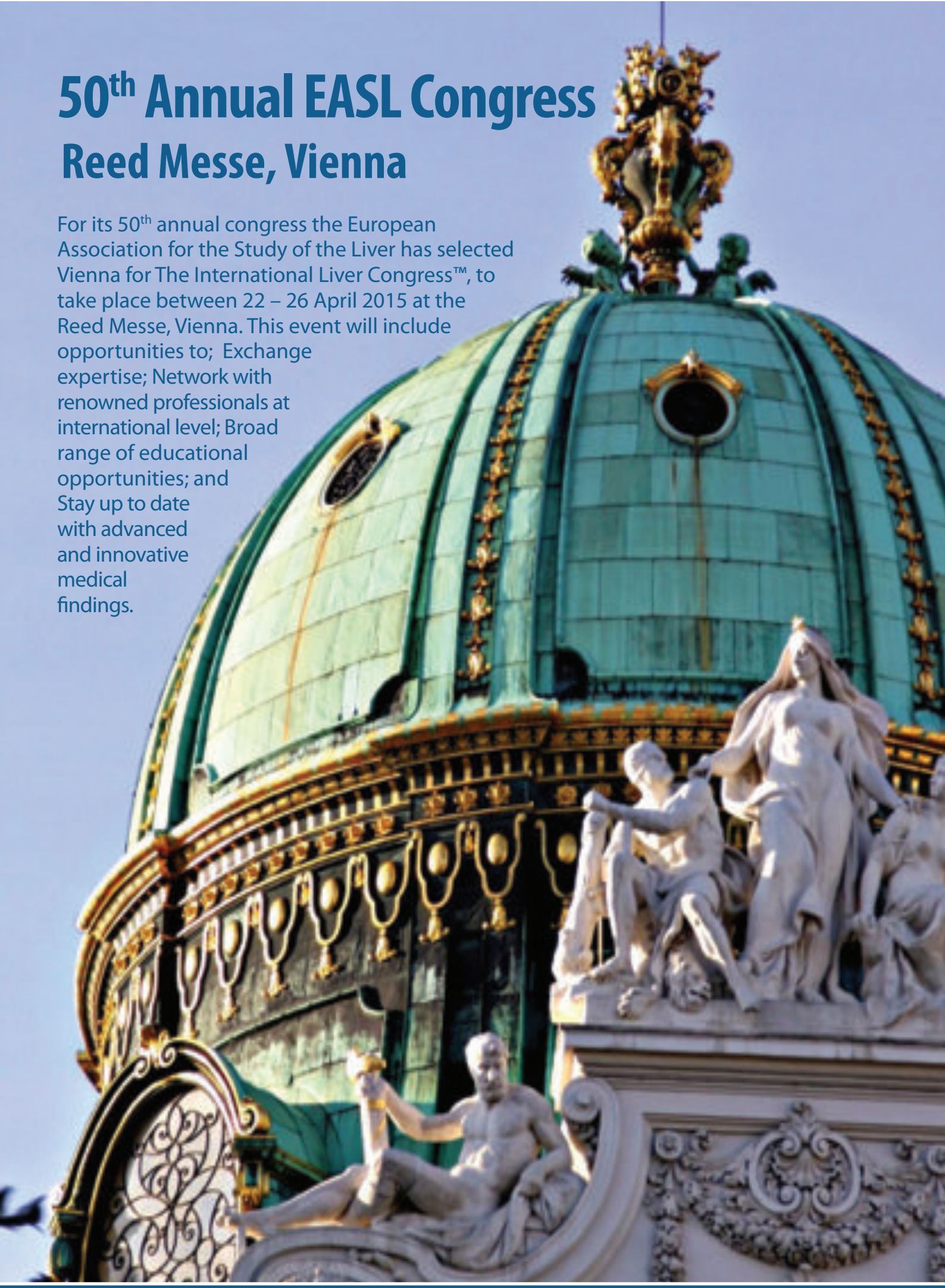
For its 50th annual congress the European Association for the Study of the Liver has selected Vienna for The International Liver Congress™, to take place between 22 – 26 April 2015 at the Reed Messe, Vienna. This event will include opportunities to; Exchange expertise; Network with renowned professionals at international level; Broad range of educational opportunities; and Stay up to date with advanced and innovative medical findings.



The Hidden World of Hepatic Encephalopathy

Summary of Presentations from the Norgine-sponsored Satellite Symposium at The International Liver Congress 2014, 49th Annual Meeting of the European Association for the Study of the Liver, London, UK, 9th – 13th April 2014

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Introduction

Hepatic encephalopathy (HE) is a common complication of liver cirrhosis that is characterised by a range of complex and variable neuropsychiatric symptoms.[1] Although the exact mechanisms underlying the pathogenesis of HE have yet to be established, the neuropsychiatric symptoms are thought to result from elevated blood levels of gut-derived neurotoxins (particularly ammonia), which enter the brain due to the inability of the cirrhotic liver to remove them from the blood.[2] Ammonia readily crosses the blood–brain barrier, where it has direct neurotoxic effects and also causes osmotic imbalance in astrocytes (the primary drivers of ammonia detoxification in the brain), causing them to swell and impairing their function.[2,3] Impairment of astrocyte function leads to a general disruption of communication in the brain, which is thought to result in the neuropsychiatric symptoms of HE.[2]

HE can be classified into three main types, based on its severity: minimal, episodic and persistent.[4-6] Minimal HE refers to patients who appear to be clinically normal, but who show slight cognitive and/or neurophysiological abnormalities. These abnormalities may only become apparent as subtle changes in reaction times during complex daily activities, such as driving, but can have a detrimental impact on health-related quality of life (HRQOL). Studies have demonstrated that over 50% of patients with cirrhosis may have minimal HE[7-9] and that many patients with minimal HE go on to develop episodic and/or persistent HE,[10] which are the clinically overt stages of the disease.

This article will focus on the serious consequences of a first overt HE episode on a patient's subsequent prognosis, the role of precipitating factors in the development of overt HE, and the therapeutic options available for early intervention and prevention of its recurrence. It will also discuss the hidden costs associated with HE, in terms of its impact on healthcare resource utilisation, and the devastating impact of HE on the quality of life (QOL) of both patients and those who care for them.

Prof. Rajiv Jalan is a Professor of Hepatology at the Institute of Liver and Digestive Health, UCL Medical School, Royal Free Hospital, UK. He has pioneered the use of hypothermia and albumin dialysis for liver failure, developed a potential new treatment option for hepatic encephalopathy and described the use of novel shunts in the treatment of variceal bleeding. Prof. Jalan's clinical and research interests are in Liver Failure and Liver Transplantation. He is the current President of ISHEN and Editor-in-Chief of *Liver International*.

Dr Mark Hudson is a Consultant Hepatologist based at the Freeman Hospital, Newcastle upon Tyne, UK. Dr Hudson is the current President of the British Association for the Study of the Liver (BASL) and also the Chair and Clinical Lead of the North East & North Cumbria Hepatology Network. He is a member of the Northern Senate Council.

Dr Jasmohan S. Bajaj is an Associate Professor of Medicine at Virginia Commonwealth University and McGuire VA Medical Center in Richmond, Virginia, USA. He 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 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.

The authors dedicate this article to the memory of **Dr Juan Cordoba**, who contributed immensely to current understanding of the burden of HE and its management, and who was to have spoken at the symposium.

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Why is the First HE Event so Important?

Professor Rajiv Jalan

It is well established that the natural history of cirrhotic disease involves the development over time of major complications, such as HE, ascites, jaundice and gastrointestinal (GI) bleeding, which drastically increase patients' morbidity and their risk of mortality.[11] The occurrence of a first major complication in a patient with cirrhosis therefore has serious implications for their subsequent prognosis and survival.

Acute-on-chronic Liver Failure

The development of one or more major acute complications of liver disease is known as acute decompensation. Patients with cirrhosis who are hospitalised for acute decompensation and organ failure are at high risk of imminent death and considered to have acute-on-chronic liver failure (ACLF).[12] The European Association for the Study of the Liver-Chronic Liver Failure (EASL-CLIF) Consortium Acute-on-Chronic Liver Failure in Cirrhosis (CANONIC) study established diagnostic criteria for ACLF and the grading of its severity.[12] The study demonstrated that patients with ACLF have a 28-day mortality rate of >25%, compared with <5% for decompensated patients with cirrhosis without organ failure.[12] The 28-day mortality risk increased sharply depending on the number of organs that failed, reaching almost 80% for patients with more than three organ failures.[12] A key factor in determining whether decompensated cirrhosis will develop into ACLF is the occurrence of a second 'hit' or event, such as infection, systemic inflammatory response syndrome or GI bleeding, which results in acute, rapid deterioration of liver function and a greatly increased risk of death. The timing of intervention(s) to support liver function in decompensated patients with cirrhosis is therefore of critical importance.[13]

The mechanisms by which decompensated cirrhosis develops into ACLF following a second 'hit' are currently unclear. One hypothesis is that endotoxaemia resulting from increased gut permeability and gut bacterial translocation in patients with cirrhosis primes the end organs via up-regulation of Toll-like receptor 4 (TLR4; a ubiquitous receptor for bacteria), and that, following infection or inflammation, ligands for TLR4 trigger an inflammatory cascade via nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which leads to organ damage and subsequent organ failure.[14] Studies conducted using the bile duct ligated rodent model have demonstrated that selective gut decontamination with norfloxacin can attenuate cerebral TLR4 expression, modulating inflammation and thereby delaying coma and improving survival in cirrhotic animals administered lipopolysaccharides.[14]

The Effect of HE on Survival

The impact of the presence and severity of HE on the survival of patients with cirrhosis with and without ACLF was investigated in sub-analyses of data from the CANONIC study.[15] Clinical, laboratory and survival data of 1348 consecutive patients with cirrhosis admitted with acute decompensation were compared according to the presence or absence of HE (n=460 and n=888, respectively) and the presence or absence of ACLF (n=301 and n=1047, respectively). HE diagnosis was defined as impairment of cognition, consciousness or motor function in a patient with cirrhosis, after exclusion of other causes of mental disturbances, and HE severity was assessed according to West Haven criteria[16] and grouped in two levels: mild (Grade I or II) and severe (Grade III or IV).[15]

Results of the sub-analyses demonstrated that mortality was significantly increased in patients with HE, compared to those without HE ($p<0.001$), particularly in those with severe HE, who often died within a few weeks of the event (Figure 1A). Overall, 43% of patients with HE died within 1 year.[15] Crucially, HE was shown to significantly increase the risk of mortality in patients with cirrhosis in both the presence and absence of ACLF, the highest mortality risk being observed in patients with HE and ACLF (Figure 1B).[15] The independent association between HE and mortality risk demonstrated in this analysis therefore indicates that HE is not merely a marker of increasing deterioration of health status in patients with cirrhosis, but rather that it may itself cause complications that further increase the risk of mortality.

Impact of Previous HE on Development of Overt HE Episodes

A key finding of the CANONIC study sub-analyses was that previous HE was the most important risk factor for the development of overt HE episodes: of the patients with HE at enrolment, 53.6% had a prior history of HE and of those with no HE at enrolment, 20.8% had a prior history of HE.[15] This difference was observed in patients with and without ACLF: among patients with ACLF, 48.1% of those with HE at enrolment had a prior history of HE, while 19.8% of those with no HE enrolment had a prior history of HE; among patients without ACLF, 56.9% with HE at enrolment had a prior history of HE, while 21.0% of those with no HE at enrolment had a prior history of HE.[15]

Role of Precipitating Factors

Factors such has active alcoholism, GI bleeding and bacterial infection can potentially precipitate the development of ACLF.[12] The original CANONIC study demonstrated that although such precipitating factors were more frequently observed in patients with ACLF (particularly in those with severe ACLF) than in those without ACLF, in over 40% of ACLF patients, no precipitating factor was observed.[12] Similarly, a number of precipitating factors have traditionally been thought to lead to the development of HE, including GI bleeding, infections, dehydration, constipation, alcohol misuse, renal and electrolyte disturbances, use of psychoactive medication (e.g. opiates, benzodiazepines), excess dietary protein (particularly animal protein), an acute deterioration of liver function and use of a transjugular intrahepatic portosystemic shunt (TIPSS).[1] However, sub-analyses of

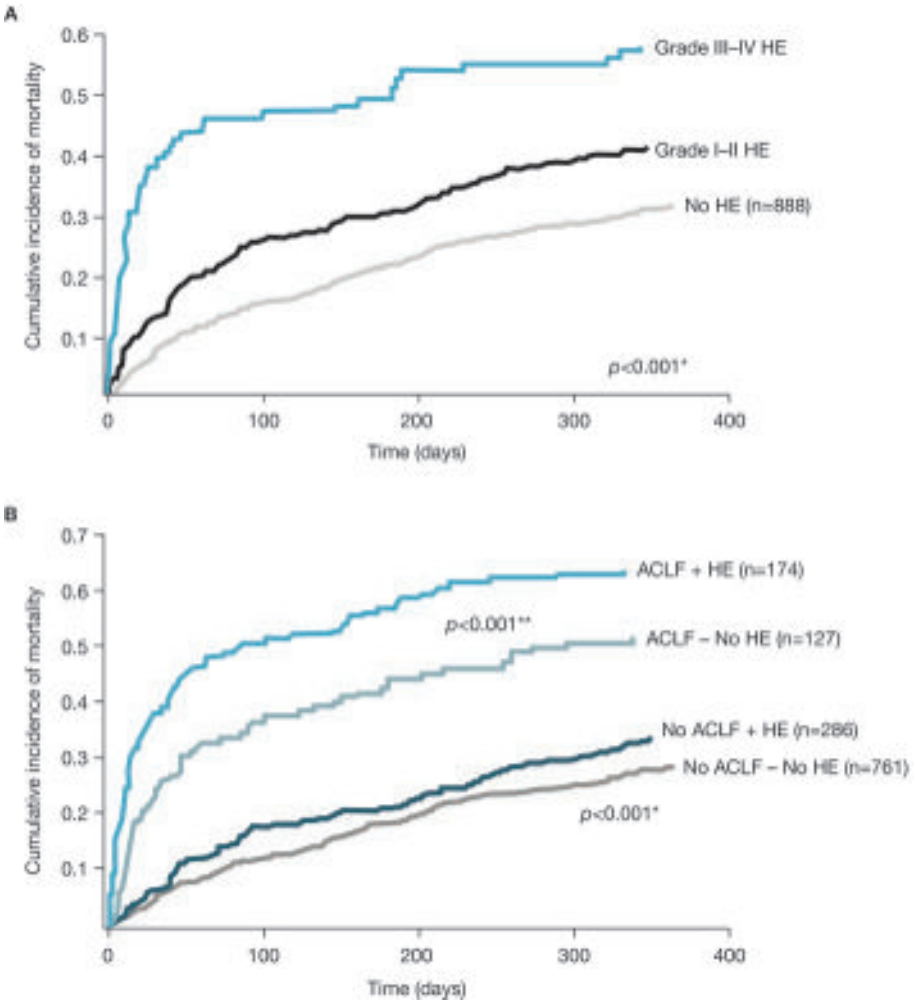

Figure 1. Sub-analyses of CANONIC study: mortality of patients with cirrhosis in relation to (A) severity of HE at inclusion (*p-value comparing all groups) and (B) the presence of HE or ACLF (alone or in combination) at inclusion (*p-value comparing presence vs. absence of HE in patients without ACLF; **p-value comparing presence vs. absence of HE in patients with ACLF). ACLF, acute-on-chronic liver failure; HE, hepatic encephalopathy. Adapted from Cordoba J, *et al.*, 2014 with permission from Elsevier.[15]

the CANONIC study found that there were no significant differences in the prevalence of bacterial infections or active alcoholism in patients with and without HE, despite these being considered to be precipitating factors for HE.[15] Similarly, the presence of TIPSS was not increased in patients with HE. The incidence of GI haemorrhage was low (perhaps reflecting improvements in the prevention and management of variceal bleeding) and significantly lower in patients with HE than in those without HE (11.1% vs. 19.3%; p<0.001). Of the 'classic' precipitating factors for HE, only hyponatraemia, renal failure and the use of diuretics were shown to be significantly higher in patients with HE, compared with those without HE.[15]

Predicting the Development of Overt HE

The serious impact of a first episode of overt HE on the subsequent prognosis and survival of a patient with cirrhosis raises the question of whether it might be possible to predict which patients with cirrhosis will develop overt HE. This question was addressed in a validation cohort study conducted by Romero-Gómez and colleagues, which demonstrated a link between the development of HE in patients with cirrhosis and specific genetic variations in the promoter region of the glutaminase gene.[17] *In vitro* functional analysis indicated that these genetic variations were associated with enhanced glutaminase transcriptional activity.[17] Patients with cirrhosis with these mutations had an approximately 40% risk of developing HE within 1 year.[17] This is thought to be the first study to show a link between a genetic defect and an organ-related complication of cirrhosis, and provides a basis for research into the possibility of using genetic screening to predict HE development in cirrhosis.

Prevention of HE

Primary Prophylaxis

Prevention of endotoxaemia is a key target for therapeutic intervention in the prevention of HE. This may be achieved either by preventing gut bacterial translocation or by targeted reduction of specific endotoxins, in particular, ammonia. The first study to demonstrate that primary prophylaxis can significantly reduce the development of a first overt episode of HE was a trial conducted in a tertiary referral institute in New Delhi, India, in which 160 patients with cirrhosis without overt HE were randomised to either receive or not receive 3 months' treatment with probiotics that are known to alter the intestinal microbiota by promoting the growth of non-urease-producing microorganisms that reduce the production of ammonia.[18] After a mean follow-up duration of approximately 40 weeks, patients who had received the probiotic treatment had significantly reduced levels of arterial ammonia, small intestinal bacterial overgrowth and oro-cecal transit time, compared with patients who did not receive the treatment. Moreover, only seven patients in the treated group developed overt HE, compared with 14 in the untreated group (p<0.05; hazard ratio [HR] for controls vs. treated group 2.1; 95% confidence interval [CI] 1.31–6.53).[18]

Secondary Prophylaxis

There is greater evidence for the effectiveness of secondary prophylaxis, since treatment with non-absorbable disaccharides, such as lactulose, and/or antibiotics, such as rifaximin-α, has been shown

to significantly reduce the risk of overt HE recurrence in patients with cirrhosis (Figure 2).[19,20]

These treatments act by reducing bacterial ammonia production and/or absorption in the intestine,[20,21] but are not specifically selective for ammonia.[22] A randomised, double-blind, Phase II trial assessed whether treatment with glycerol phenylbutyrate (GPB) can specifically lower ammonia levels in patients with cirrhosis and thereby reduce the recurrence of HE.[22] The trial was conducted in 178 patients with cirrhosis who had experienced two or more overt HE events in the previous 6 months. After 16 weeks, GPB treatment, compared with placebo, was shown to significantly reduce the proportion of patients who experienced an HE event (21% vs. 36%, p=0.02) and the time to the first event (HR 0.56, p<0.05).[22] Plasma ammonia levels were significantly reduced in patients treated with GPB versus placebo (p=0.04) and there was a significant correlation between the odds of a patient experiencing an HE event and their ammonia level, whether assessed at baseline (p=0.01) or during the study (p=0.01).[22]

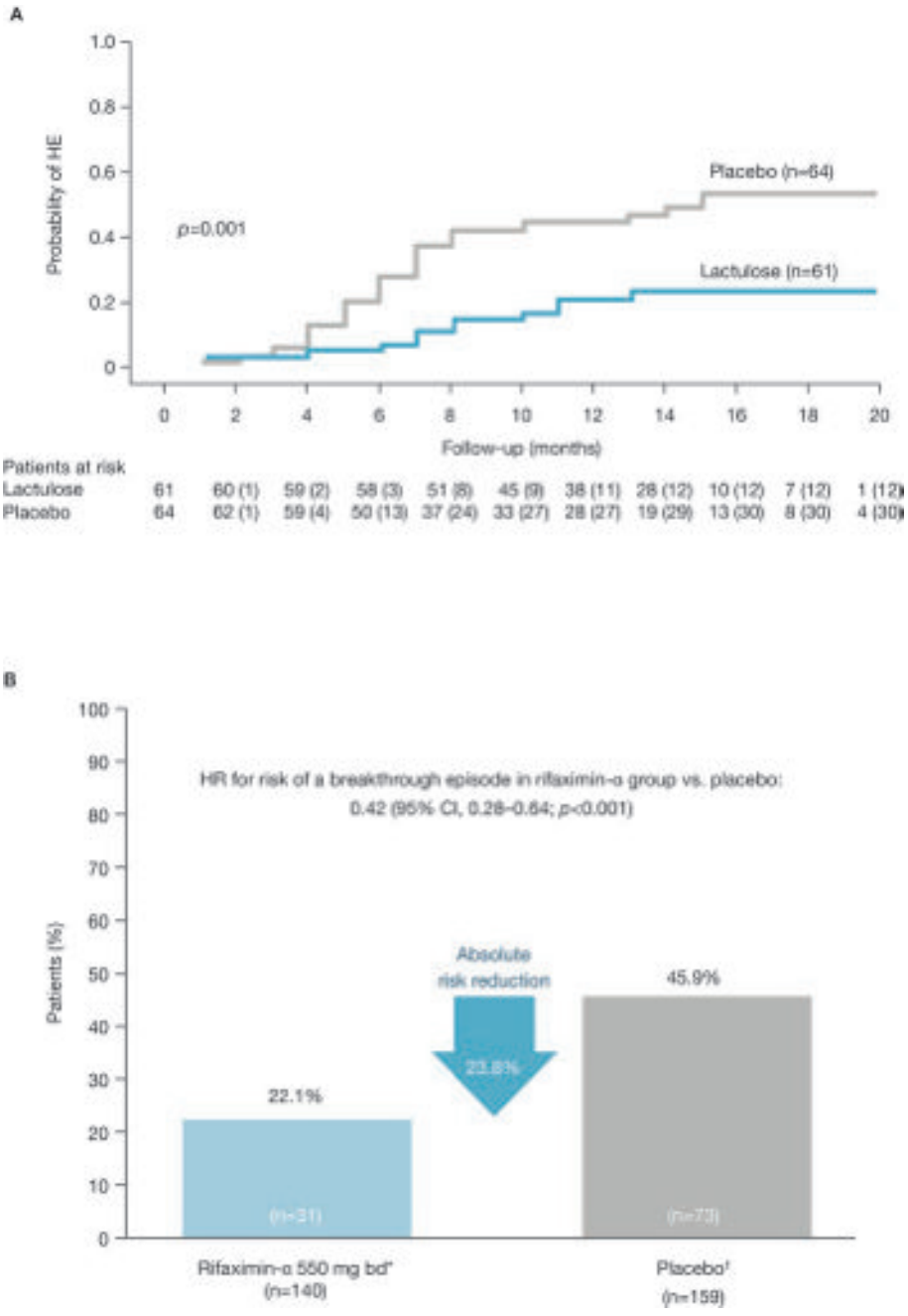

Figure 2. Effect on prevention of recurrence of HE in patients with cirrhosis of treatment with (A) lactulose and (B) rifaximin-α. *91.4% on concomitant lactulose; †91.2% on concomitant lactulose. bd, twice daily; CI, confidence interval; HE, hepatic encephalopathy; HR, hazard ratio. Adapted from (A) Sharma BC, *et al.* 2009[19] with permission from Elsevier and (B) Bass N, *et al.*, 2010.[20]

In summary, HE drastically alters the natural history of disease in cirrhosis. The occurrence of the first overt HE episode is an important hidden problem, since it is associated with a significant increase in the risk of mortality, in patients both with and without ACLF. The pathophysiological mechanisms underlying this process appear to involve inflammation and ammonia, both of which are important targets for treatment. Since overt HE can develop in the absence of 'traditional' precipitating factors, the success of potential primary prophylaxis strategies in the future will depend on the reliable identification of patients at high risk of developing HE. Initial findings suggest that screening for genetic mutations in the glutaminase gene may provide a useful tool for identifying such patients, although further confirmatory research and cost-effectiveness studies are required. Targeting the gut with lactulose and/or antibiotic treatment to reduce the recurrence of HE is widely established. In addition, emerging strategies, including primary prophylaxis with probiotics and secondary prophylaxis targeting the specific reduction of plasma ammonia levels, have shown promising results. In conclusion, occurrence of a first episode of overt HE is associated with significant mortality and morbidity, warranting strategies targeting its prevention and appropriate early management.

The Hidden Costs of Managing HE and Emerging Evidence on the Impact of HE on Mortality

Dr Mark Hudson

Overt HE is characterised by clinically evident neurophysiological or neuropsychometric changes that often require hospitalisation. This can be frequent, expensive to manage and have a detrimental impact on patients' QOL.[23]

Clinical Practice Research Datalink Study

Comparatively little research into the epidemiology of HE and its association with adverse outcomes has been undertaken to date. A study was conducted in the UK using data from the Clinical Practice Research Datalink (CPRD), which aimed to detail the epidemiology of chronic liver disease, with a specific focus on HE, and to determine whether HE is associated with different patterns of mortality and resource use. The CPRD is a longitudinal, anonymised research database derived from over 650 primary care practices in the UK,[24,25] containing clinical records from more than 13 million people and providing a good representation of the UK population in terms of geographical spread. The study was conducted using a subset of CPRD data from practices based in England that are linked to additional health-based datasets, including the Hospital Episode Statistics (HES) dataset.[24,25] Patients with a first diagnosis of liver disease between 1st January 1998 and 31st December 2012 were identified by Read code from the CPRD dataset and by International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) code[26] from the linked HES data.[24,25] Patients with HE were diagnosed according to Read code J622.00 and J622.11.[24,25] For comparative analyses, patients with HE were matched to liver disease patient controls at a ratio of 1:1 on the basis of age, sex, calendar year of first diagnosis, duration of liver disease (±90 days) and grade of cirrhosis compensation/decompensation.[24,25] The level of compensation/decompensation was graded using the Baveno IV classification system.[27] Rates of primary care consultations were calculated and inpatient activity was assessed using standardised admission ratios. In total, 17,030 patients with a first diagnosis of liver disease were identified, not all of whom had cirrhosis. Of these, 551 (3.2%) were diagnosed with HE, all of whom had cirrhosis. Overall, 389/551 (70.6%) patients with HE could be matched 1:1 to non-HE controls.[24,25]

A key finding of the study was that the risk of mortality was significantly increased in patients with HE compared with those without HE (p=0.000; Figure 3).[24] Of the patients identified with HE for whom matched controls were available, 226/389 (58.1%) died during the follow-up period, compared with 126/389 (32.4%) of the matched controls, corresponding to a crude relative mortality risk of 2.73 (95% CI 2.20–3.41).[24] In a Cox proportional hazards model using the matched controls, the HR for death for patients with HE versus controls was 2.28 (95% CI 1.82–2.87).[24] These findings demonstrated that the risk of death in patients with cirrhosis with overt HE was more than twice that of patients with cirrhosis with the same level of compensation/decompensation who did not have overt HE.[24]

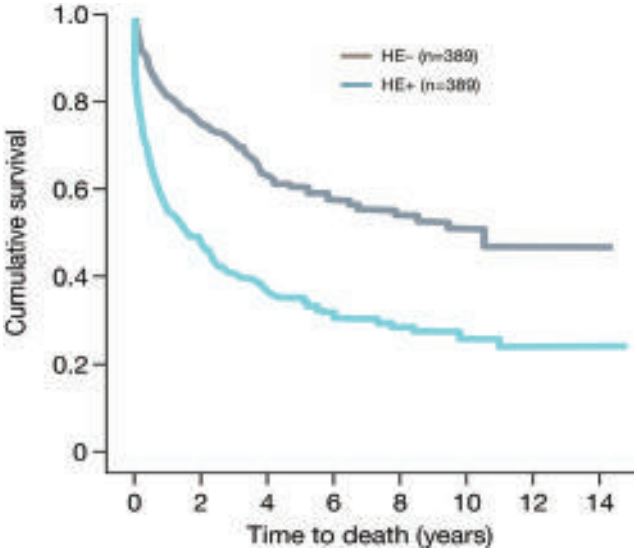

Figure 3. Kaplan Meier curve for time to death in patients with cirrhosis with and without HE. HE, hepatic encephalopathy. Adapted from Morgan CL, *et al.*, 2014.[24]

Analysis of healthcare resource use revealed that patients with HE had a significantly greater number of hospital admissions than those without HE: crude admission ratios were 3.59 (95% CI 3.33–3.87) for admissions with primary diagnoses of liver disease and 3.05 (95% CI 2.91–3.19) for any diagnosis of liver disease.[25] Interestingly, patients with HE were also shown to have a significantly greater number of hospital admissions for non-liver related diagnoses (admission ratio 1.46; 95% CI 1.36–1.57),[25] providing an indication of the wider impact of HE; for example, in terms of falls and other accidents resulting from having the condition. The length of stay in hospital was similar for patients with HE compared with those without HE (mean [SD] length of stay: 8.0 [11.6] vs. 6.8 [9.5] days, respectively; p=0.148), but there was a significantly higher proportion of liver-related emergency admissions among patients with HE, compared with patients without HE (62.1% vs. 50.0%; p<0.001).[25] Following their first HE event, patients with HE had 18.2 primary care contacts per patient year compared with 8.7 for non-HE controls (p<0.001).[25] Given the significantly higher rate of liver-related emergency admissions associated with HE, these primary care data perhaps indicate that greater awareness of, and intervention for, HE in the primary care setting could help reduce the number of HE-related emergency hospital admissions and the associated costs of hospitalisation. Overall, the CPRD study demonstrated that, in patients with chronic liver disease, HE was not only associated with a significant increase in the risk of death, but also with a significantly increased burden in terms of healthcare resource utilisation.[24,25]

North East and North Cumbria Liver Network Audit on Cost-effectiveness

The cost implications of increased healthcare resource utilisation associated with the management of HE have driven research into the

cost-effectiveness of treatments targeting the condition. Rifaximin-α is an oral antimicrobial agent with broad-spectrum activity that is used for the reduction in recurrence of episodes of overt HE.[28] The North East and North Cumbria Liver Network Audit on Cost-effectiveness study evaluated the cost-effectiveness of rifaximin-α treatment in reducing emergency hospital admission during 1 year of treatment.[29] The audit included data on patients starting rifaximin-α treatment at the Freeman Hospital, Newcastle upon Tyne, UK, between January and December 2011, identified from pharmacy records. For patients who were alive after 1 year, the number and length of emergency hospital admissions were compared for the periods 1 year prior to starting and 1 year after starting rifaximin-α treatment. The cost-effectiveness of rifaximin-α treatment was calculated using the standard British National Formulary (BNF) tariff for rifaximin-α (based on BNF May 2013 prices) and the estimated average cost per day for acute inpatient medical admission to the Newcastle upon Tyne hospitals (£350/day).[29]

In total, 64 patients started rifaximin-α treatment for HE (48 male, 16 female), of whom 40 (62.5%) were taking concomitant lactulose.[29] Rifaximin-α was stopped in nine patients, due to the patient undergoing liver transplantation (n=5), HE being excluded (n=3) and side effects (tiredness and lethargy; n=1). Twenty-three patients died within 1 year, the Model for End-Stage Liver Disease (MELD) score being significantly higher for these patients than for the survivors (19.0 vs. 13.0; p<0.05). Thirty-two patients were alive and remained on rifaximin-α at 1 year and complete data were available for 27 of these patients.[29] Results of the audit demonstrated that the mean (SD) number of emergency admissions was significantly reduced from 2.7 (2.4) per patient for the year prior to starting rifaximin-α treatment to 1.8 (2.9) per patient in the year of treatment (p<0.05).[29] Similarly, the mean (SD) duration of inpatient admissions was significantly reduced from 28.4 (26.3) bed days per patient for the year prior to starting rifaximin-α treatment to 9.4 (14.7) bed days per patient in the year of treatment (p<0.05).[29] When the cost of 1 year of treatment with rifaximin-α was taken into account, the reduction in the number and duration of emergency admissions represented a mean annual saving of £2964 per patient (Figure 4).[29] This audit therefore demonstrated that rifaximin-α reduced emergency hospital admissions when prescribed for the reduction of recurrence of HE and that, at this centre, treatment with rifaximin-α for secondary prevention of HE was cost-effective for reducing emergency admission to hospital.[29]

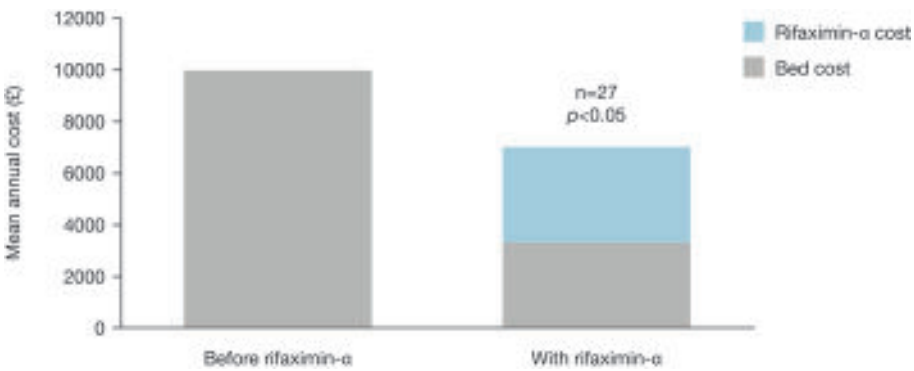

Figure 4. North East and North Cumbria Liver Network Audit on Cost-effectiveness: mean annual costs per patient pre and post treatment with rifaximin-α. Adapted from Orr J, *et al*., 2013.[29]

In summary, HE has a significant impact on resource utilisation by healthcare systems. HE has been shown to be associated with a greater number of hospital admissions, length of stay in hospital and GP contacts. A greater awareness and intervention for HE in the primary care setting may therefore help to reduce subsequent hospitalisation costs. Together with the increased mortality risk associated with the condition, the significant economic burden of HE supports the need for strategies to reduce recurrences of breakthrough episodes.

The Lost Patient: a QOL Case Study

Dr Jasmohan S. Bajaj

Liver cirrhosis and HE place a serious burden on patients and also on their carers and families. Patients with HE have a high risk of death and hospitalisation, and the condition also has important psychosocial implications, decreasing patients' QOL and increasing their dependence on caregivers.[15, 30, 31] Multiple aspects of a patient's life are affected by the condition, including their social interaction with friends and family, sleep pattern, sexual activity and ability to drive.[1,32,33] Additionally, HE has a negative impact on patients' financial status, since it affects their work performance and employment prospects.[31] It is, however, important to recognise that the health, financial status and psychosocial condition of those caring for a patient with HE can also be severely affected as a result of the condition,[31,34] as illustrated in the case study presented in Figure 5.

Measurement of HRQOL

Although definitions of HRQOL vary, it is generally accepted to comprise three core domains: psychological functioning (well-being and emotional status), social functioning and physical functioning.[35] HRQOL can be assessed using generic and/or disease-specific instruments. Generic instruments include the Short Form 36 Health Survey (SF-36),[36] the Sickness Impact Profile (SIP)[37] and the Nottingham Health Profile.[38] Examples of disease-specific instruments for chronic liver disease include the Chronic Liver Disease Questionnaire (CLDQ), [39] the Liver Disease Quality-of-Life Questionnaire (LDQOL),[40] the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)'s Quality-of-Life Questionnaire[41] and the

- A 55-year-old army veteran with hepatitis C and alcohol-related cirrhosis was seen with his wife for a routine visit.
- His past medical history showed two episodes of overt HE within the previous 4 months, the first due to spontaneous bacterial peritonitis, the second to non-adherence to lactulose treatment.
- Although the patient himself did not offer any specific complaints, upon further questioning his wife stated that he was "feeling down". He was also getting tired very easily, could not move or get up easily, had poor sleeping habits and was forgetting things easily.
- On the day of the visit, the patient's MELD score was 12 and his Child-Turcotte-Pugh score was 9.
- The patient underwent testing for cognitive function (using matrix reasoning) and HRQOL (using the SIP and PROMIS) and was found to be severely impaired on both.
- When questioned further about why he had been feeling down, the patient was reluctant to give reasons, but his wife said that, due to his advanced liver disease and HE, her husband was forced to give up his job as a machine tool operator, having had several accidents.
- The patient stated that this had severely reduced their ability to meet household expenses, which had made him feel "less of a man" and depressed.
- The patient's wife had also been required to cut down her work hours due to her husband's illness, including the need to drive him back and forth to medical appointments, since he had been advised not to drive due to his condition.

Figure 5. A QOL case study. HE, hepatic encephalopathy; HRQOL, health-related quality of life; MELD, Model for End-Stage Liver Disease; PROMIS, Patient-Reported Outcomes Measurement Information System; QOL, quality of life; SIP, Sickness Impact Profile.

Primary Biliary Cirrhosis 40.[42] In the USA, the National Institutes of Health-sponsored Patient-Reported Outcomes Measurement Information System (PROMIS) instrument is increasingly being used.[43] The use of validated tools such as these provides a means of assessing subtle changes in HRQOL, which enables clinicians not only to accurately gauge how their patients are feeling and functioning, but also to assess how effective a treatment is on those aspects of life that are most relevant and important to the patients themselves. As such, it has become increasingly recognised that HRQOL is not a 'soft outcome', but, rather, a key parameter for measuring the effectiveness of disease management strategies, in both clinical practice and research settings.

Impact of HRQOL and Socioeconomic Status on Survival

Many studies using generic and/or disease-specific instruments have demonstrated that HE impairs daily functioning and is associated with impaired HRQOL.[43-50] Importantly, HRQOL has been shown to independently predict mortality in patients with advanced liver disease when all other factors (e.g. MELD score) have been taken into account, lower HRQOL being associated with higher mortality (Figure 6).[51]

Survival of patients with liver cirrhosis has also been shown to be associated with socioeconomic status. For example, a Danish population cohort study of >1700 patients with liver cirrhosis demonstrated that patients' socioeconomic status deteriorated during the 5 years preceding their cirrhosis diagnosis, and that 5-year survival was associated with socioeconomic status.[52] Specifically, married patients had a better survival rate than those who were divorced or who had never married, and patients who were disability pensioners had a poorer survival rate than those who were employed or unemployed.[52]

Impact of HE on the HRQOL of Patients

Psychometric analysis has demonstrated that minimal HE primarily affects psychomotor function but not verbal abilities.[45] Consequently, the condition appears to have a greater impact on the ability to work and/or drive in 'blue collar' workers than in 'white collar' workers: one study found that 60% of 'blue collar' workers with minimal HE were deemed unfit to work, compared with 20% of 'white collar' workers with the condition.[45] Several studies have demonstrated that patients with HE – including those with minimal HE – are likely to be unsafe drivers.[53-55] Importantly, patients with minimal HE may not be aware that their work performance and ability to drive are impaired.[33] It is therefore particularly important that the driving ability of patients with cirrhosis be routinely assessed, in order to decrease motor vehicle accident rates.[33,54] Research has also indicated that the impact of HE on HRQOL varies between men and women: an Italian study that used the Nottingham Health Profile to measure HRQOL found that the greatest impairments for male patients were in their paid employment and sex life, while, for female patients, the greatest impairments were in their social life and home life.[56]

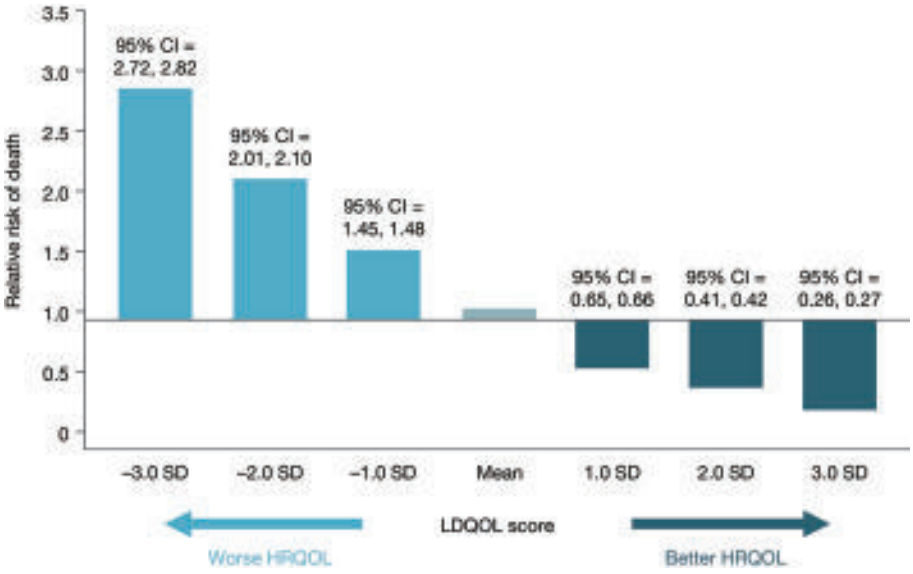

Figure 6. Association between liver disease-targeted HRQOL and short-term mortality. CI, confidence interval; HRQOL, health-related quality of life; LDQOL, Liver Disease Quality-of-Life Questionnaire; SD, standard deviation. Adapted from Kanwal F, *et al.*, 2009 with permission from Elsevier.[51]

A cross-sectional study of >100 patients with cirrhosis and their caregivers demonstrated that cirrhosis severely impacted the patient's family unit, in terms of employment, financial status and caregiver burden.[31] Patients with previous HE had significantly worse unemployment (88% vs. 19%; p=0.0001), were significantly more likely to have to reduce their work hours (71% vs. 39%; p=0.017) and were significantly more likely to have a poor financial status (85% vs. 61%; p=0.019) than those without previous HE.[31] The baseline MELD score of patients with previous HE was significantly worse than those without previous HE (15.5 vs. 10.7; p=0.00001), and cognitive performance and MELD score were significantly correlated with employment status.[31] The study also demonstrated that cirrhosis-related expenses had a detrimental impact on almost all areas of life for the patient's family unit (Figure 7).[31] Similarly, in an international, multicentre, cross-sectional study of >200 patients with cirrhosis, cognitive function was shown to be strongly associated with socioeconomic status (based on employment and personal income), independent of country, age or education.[57] When patients with a prior history of overt HE were compared with those without such a history, they were shown to have significantly worse cognitive performance overall, with higher rates of unemployment and lower personal income.[57]

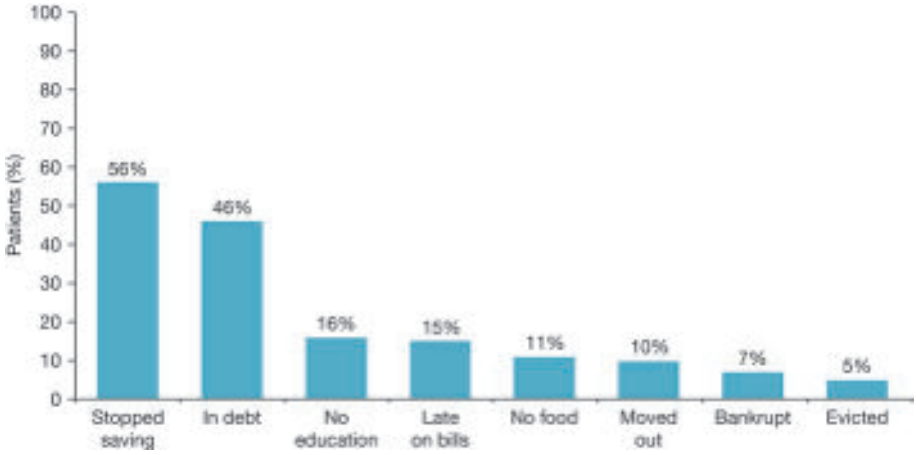

Figure 7. Impact of cirrhosis-related medical expenses on daily activities of the affected family within the last 3 years. No education: a family member's education suffered; late on bills: late on utility bills, rent and car payments; no food: had to skip food; moved out: needed to move to a cheaper place; bankrupt: had to declare personal bankruptcy. The percentage of patients who responded 'yes' is shown in the bar graphs. Adapted from Bajaj JS, *et al*., 2011 with permission from Macmillan Publishers Ltd.[31]

Impact of HE on the HRQOL of Caregivers

The impact of cirrhosis and HE on the lives of patients' caregivers has been specifically investigated in several studies.[31,58-61] In one investigation, patients with previous HE were shown to pose a significantly higher burden on caregivers than those without previous HE, as assessed using the Perceived Caregiver Burden score and Zarit Burden Interview-Short Form (p<0.05 for both).[31] In particular, caregivers reported that caring for a patient with HE affected their personal schedule (p=0.005) and their health (p=0.006), and gave them a feeling of entrapment (p=0.016), since they were unable to plan any activities.[31] Another study demonstrated that caregiver burden increases as patients' neuropsychiatric performance worsens, being greatest for carers of patients with overt HE .[61]

HRQOL as a Target for Treatment

It is therefore important to effectively treat and manage HE, not only to improve patients' outcomes in terms of mortality and morbidity (thereby also decreasing the associated economic burden placed on healthcare systems), but also to improve the HRQOL of patients and their caregivers. This requires a multidisciplinary approach to patient care – both inpatient and outpatient – that includes psychological input and social work, while focusing on the family unit.[31] Treatment with non-absorbable disaccharides, such as lactulose, and/or antibiotics, such as rifaximin-α, has been shown to significantly reduce the risk of overt HE recurrence in patients with cirrhosis (Figure 2).[19,20] Moreover, studies have demonstrated significant improvements in HRQOL (as assessed using the SIP) in patients with minimal/covert HE treated with lactulose (p=0.002) or rifaximin-α (p=0.000).[47,50] Importantly, remission from overt HE with rifaximin-α treatment has been shown to result in significant improvements in HRQOL, as assessed using the CLDQ, when compared with patients who subsequently experienced a breakthrough episode of HE, both in terms of the overall CLDQ score (p<0.0001) and the individual domain scores for fatigue, abdominal symptoms, systemic symptoms, activity, emotional function and worry (p<0.0001 for all).[23] It is noteworthy that the impairments in HRQOL that patients in this study who experienced a breakthrough HE episode reported preceded the occurrence of the breakthrough HE episode.[23] Rifaximin-α has also been shown to significantly improve driving simulator performance in patients with minimal HE, compared with placebo (p=0.013).[62]

In summary, the deleterious effects of HE on HRQOL can have a devastating impact not only on the lives of patients, but also on the lives of those who care for them. Effective management of HE requires a multidisciplinary approach to care that addresses the needs of the individual patient, both in the hospital setting and once they are discharged into the community, and which additionally provides the necessary support structure for their caregivers and families. Such individualised, multidisciplinary care can not only improve patients' clinical outcomes, but may also result in important improvements in the day-to-day lives of both patients and their caregivers.

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Chronic Pancreatitis and Pancreatic Insufficiency in Chronic Alcoholic Liver Disease

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Introduction

Chronic alcohol consumption is a major cause of morbi-mortality all around the world. Alcohol affects every organ and causes a variety of disorders. Liver damage ranges from liver steatosis to cirrhosis. Although the most common cause of chronic pancreatitis has always been alcohol, others factors have also been studied, such as smoking. It is unknown how many years of alcohol intake are needed to produce pancreatic damage, but it is estimated to be less than that required to produce liver damage.

Most epidemiological studies conducted between 1960 and 2000 pointed to alcohol as the main cause of this disease, reporting that habitual consumers of alcohol accounted for 70% to 80% of patients.[1-6] More recently, doubts have been cast on the role of alcohol as a cause of chronic pancreatitis, with some studies reporting it to be responsible for only 34% to 44% of cases.[7-9] Controversy also exists regarding the association between duration of alcohol intake and the development of chronic pancreatitis and liver disease.[10-12]

Chronic Pancreatitis and Pancreatic Insufficiency

Chronic pancreatitis is characterised by progressive damage that may impair the endocrine and exocrine functions of the gland; this damage is associated with the progressive development of both ductal and parenchymal histopathological and morphological changes in the exocrine and endocrine functions.[13]

There are no clearly defined laboratory parameters for the diagnosis of chronic pancreatitis. Some patients present with non-specific abdominal pain as the only manifestation. At advanced stages, patients may experience exocrine insufficiency and diarrhoea or changes in bowel habits with or without weight loss. In these cases, diagnosis can be made either by directly quantifying the pancreatic enzymes in the duodenal juice after hormonal intake or stimulation, or by measuring the substrates produced by inadequate pancreatic enzyme digestion. In the most severe cases, endocrine failure is manifested as diabetes mellitus.

The morphological diagnosis of chronic pancreatitis requires images — whether simple abdominal X-rays, abdominal ultrasound, computed tomography, magnetic resonance or endoscopic retrograde cholangiopancreatography. As the most sensitive diagnostic method, endoscopic ultrasonography is the diagnostic tool of choice.[14] To test for exocrine pancreatic insufficiency, the breath test is gradually replacing tests like faecal elastase measurement, given its easy implementation and high diagnostic sensitivity.[15] This test obtains close to 90% sensitivity and specificity for maldigestion secondary to exocrine pancreatic insufficiency.[13] Patients are asked not to eat corn or fibre-rich products the day before the test, to have breakfast at least 2 hours before the test, not to smoke in the 6-8 hours before or during the test and not to do physical exercise. For the test, patients ingest two small pieces of toast and 20 g of butter with a substrate mixture of 250 mg of 13C mixed-triglyceride in powder form, 200 mL of water and 10 mg of metoclopramide. A baseline breath sample is taken at 20 minutes and thereafter every 30 minutes for 6 hours (12 measurements in total). Duodenal hydrolysis induced by pancreatic lipase causes the labelled metabolites to be absorbed and metabolised by the liver, and $13CO_2$ is expelled in the breath. Infrared spectrophotometry is used for the analysis and a mathematical programme calculates the percentage substrate recovered.

Alcohol Liver Disease

Chronic alcohol consumption is associated with varying degrees of liver disease, ranging from simple fatty liver (steatosis) to confirmed cirrhosis. Alcohol is metabolised in the liver via the cytosolic dehydrogenases, cytochrome P450 and peroxisomes.[16] Liver damage occurs as a result of alcohol oxidation in this organ and of an imbalance in the redox mechanisms, as well as a consequence of increased oxidative stress caused by the same oxidation process or due to an absence or deficiency of certain antioxidants. Although there is a close link between the amount and duration of alcohol intake and the occurrence of hepatic lesions, only around 30% to 40% of alcohol users have clinically significant liver damage.[17]

Pancreatic Insufficiency, Chronic Pancreatitis and Chronic Liver Disease: Coincidence or Shared Toxicity?

Few studies have analysed the prevalence of pancreatic insufficiency and chronic pancreatitis in patients with liver disease. Using the faecal elastase test, Aparisi *et al*.[18] calculated that 7% of patients with cirrhosis had pancreatic insufficiency, compared to 14.8% of asymptomatic alcoholic patients.

It has been estimated that fewer than 5% of alcohol-consuming patients develop chronic pancreatitis.[19-21] Previous studies have indicated cirrhosis to be present in between 5% and 30% of patients diagnosed with chronic pancreatitis.[22] Angelini *et al*.[11] found that 12.5% of patients with chronic alcoholic pancreatitis also had findings for liver cirrhosis, but did not associate histological findings for the liver (extent of fibrosis) with the severity of pancreatic insufficiency.

The Aparisi[18] study mentioned above, which used the faecal elastase test to diagnose exocrine pancreatic insufficiency and the indocyanine green clearance test to diagnose liver failure, pointed to an inverse relationship between hepatic and pancreatic function in patients with chronic alcoholic pancreatitis and cirrhosis. The authors, concluding that alcohol usually damages a single organ, found no association between insufficiencies in the two organs.

In the same vein, Hayakawa *et al*.[22] reported a negative correlation between liver and pancreatic function in alcohol consumers diagnosed with chronic liver disease. The same authors also observed increased pancreatic secretion in patients with alcoholic liver disease.

A number of studies have pointed to the existence of hypersecretory states for pancreatic juice — as measured by the secretin test — in patients with chronic alcoholic liver disease.[23, 24] This pancreatic juice contained less protein and calcium and so protected the pancreas from the formation of protein plugs and calcifications.[22, 25] Dreiling *et al*.[26] formulated the hypothesis regarding the hypersecretory pancreatic state in cases of alcoholic cirrhosis, mentioning the possibility that a hypersecretory state in alcohol users with liver disease may be due to reduced inactivation of secretin or an outcome of portal hypertension.[22, 23] The hypersecretory state has also been encountered in animal models; low protein levels were found in the pancreatic juice of rats that were administered high amounts of alcohol, a situation which reversed after the alcohol was withdrawn.[24] The same study found that chronic alcohol intake altered neurohormonal control of the pancreas, especially in central sites involving the extreme area and at the acinar cell level with large quantities of alcohol. Chronic alcohol intake in large amounts was associated with pancreatic protein hypersecretion mediated by central vago-vagal disinhibition pathways and also occurring at the acinar cell level. The suspension of alcohol intake partially reversed the neurohormonal dysregulation of the liver function. These data support a model of rats that are chronically administered high doses of alcohol and which have an enhanced susceptibility to hyperstimulation and hypersecretion, especially during the abstinence phase. Hyperstimulation combined with alcohol-induced lowering of the acute pancreatitis threshold may combine to trigger acute pancreatitis episodes in alcoholics and may eventually lead to chronic pancreatitis.[24]

Sakai *et al*.[27] who administered the cholecystokinin-pancreozymin test, concluded that the prevalence of pancreatic insufficiency was greater in patients with chronic liver disease of any etiology and that there were no differences between patients with and without cirrhosis.

The medical literature includes no studies based on using the mixed-triglyceride breath test to detect exocrine pancreatic insufficiency, and furthermore, includes few studies that distinguish between patients with and without cirrhosis.

Few studies have evaluated the existence of chronic pancreatitis and pancreatic insufficiency in a population with chronic liver damage due to alcohol intake.[12, 18] Our working group[28] determined the prevalence of exocrine pancreatic insufficiency and chronic pancreatitis in patients with chronic liver disease caused by alcohol, and assessed the factors possibly associated with their development. In this observational study, patients with alcoholic liver disease were compared with a group of subjects with chronic non-alcoholic liver disease. All patients were asked to perform the 13C mixed-triglyceride breath test. Patients who reported abdominal pain were evaluated for chronic pancreatitis by endoscopic ultrasonography in accordance with diagnostic criteria as defined by Wiersema[29] (four or more criteria determine a diagnosis of chronic pancreatitis). Indeterminate or suggestive cases of chronic pancreatitis were excluded. This study demonstrates a high prevalence of pancreatic insufficiency (55.2%) and chronic pancreatitis (44%) in subjects with chronic alcoholic liver disease. Pancreatic insufficiency appears to be more common in the early stages of liver disease, as demonstrated by findings of lower prevalence in patients with cirrhosis (46.2% vs. 70%, $p = 0.017$) and is inversely associated with liver failure (albumin, INR, prothrombin time and thrombocytopenia) and portal hypertension (ascites, splenomegaly and gastroesophageal varices) parameters. These findings have not previously been reported in studies that do indicate that alcohol usually only affects a single organ (either liver or pancreas).[18, 30] The fact that a pancreatic hypersecretion state has been described in patients with alcoholic liver disease[22-24, 26] may explain the finding that patients with cirrhosis have a lower prevalence of pancreatic insufficiency compared to those without cirrhosis. It is widely accepted that alcohol is a risk factor for chronic pancreatitis.[17] The strong correlation existing between chronic pancreatitis and abdominal pain would tend to lead to a suspicion of this disorder in patients with this symptom, so such patients should undergo endoscopic ultrasonography once other possible

causes of abdominal pain are ruled out. This study is the first that used the 13C mixed-triglyceride breath test as a diagnostic method for exocrine pancreatic insufficiency in alcoholic liver disease patients, unlike previous studies, which have analysed faecal elastase, directly obtained pancreatic juice and urinary n-benzoyl-tyrosyl-para-aminobenzoic acid.

By improving clinical, laboratory and nutritional parameters, pancreatic supplements may play an important role in the treatment of patients with pancreatic insufficiency. However, more studies are needed to assess the benefit of diagnosis and treatment of pancreatic insufficiency in this population.

In conclusion, patients with chronic alcoholic liver disease seem to have a higher prevalence of pancreatic insufficiency and chronic pancreatitis —most especially patients who have not yet developed cirrhosis with liver insufficiency or portal hypertension. In our study, liver function and portal hypertension parameters were inversely correlated with pancreatic insufficiency and chronic pancreatitis. Abdominal pain was frequently associated with an endoscopic ultrasonography finding of chronic pancreatitis and was present in patients with chronic non-cirrhotic liver disease. Patients without cirrhosis frequently had more episodes of acute pancreatitis. In our study, we found no association between smoking and chronic pancreatitis and so we did between diarrhoea and pancreatic insufficiency.

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The Mechanism of Cholesterol Gallstones Formation and Strategy of their Nonsurgical Therapy

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Gallstone disease (GD, cholelithiasis) is a chronic recurrent hepatobiliary disease, the basis for which is the impaired metabolism of cholesterol, phospholipids, bile acids and bilirubin, which is characterised by the formation of gallstones in the hepatic bile duct, common bile duct or gallbladder.[1] Cholelithiasis is one of the most prevalent gastrointestinal diseases, with a substantial burden to health care systems[2] and economically relevant health problems of industrialised countries and urbanised cities.[3] Gallstone disease is a common disorder all over the world[4] and can have serious outcomes, such as acute gallstone pancreatitis and gallbladder cancer. The prevalence varies widely by region from 5.9-21.9% in Western society to 3.1-10.7% in Asia.[5]

Most gallstones (75%) are silent.[5] In past decades, there has been a tendency toward the increased incidence of GD. It can be explained by better diagnosis and by the new noninvasive ultrasound techniques being introduced into clinical practice. Also of importance is a change in lifestyle[6]: reduction of motor activity, reduction of the physical load and changes to diets. One of the important benefits of early screening for gallstone disease is that ultrasonography can detect asymptomatic cases, which results in early treatment and the prevention of serious outcomes.[2,7]

Gallstones (GS) in the gallbladder and/or bile ducts are a morphological substrate of GD. The major components of virtually all types of GS are free unesterified cholesterol, unconjugated bilirubin, bilirubin calcium salts, fatty acids, calcium carbonates and phosphates, and mucin glycoproteins. Three main categories of gallstones can be identified according to their predominant chemical composition: cholesterol, pigment and mixed stones.[8] Cholesterol stones account for more than 75% of all gallstones in GD.[4,9]

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This review will discuss the mechanisms of the cholesterol gallstones (ChGS) formation, their composition and nonsurgical methods of treatment.

ChGS are composed of about 70% cholesterol monohydrate.[10] Color cathodoluminescence scanning electron microscopy (CCLSEM) studies of ChGS (Figure 1) have shown that their major components are cholesterol (Figure 1A) and glycoproteins (Figure 1B) constituents. Bilirubin is arranged as individual embedments onto the surface of the section of a stone (Figure 1C).[11,12]

The pathogenesis of GD is suggested to be multifactorial and probably develops from complex interactions between many genetic[5,6] and environmental factors.[2,13,14] Cholesterol gallstones are formed in the gallbladder due to impaired relationships between the major bile components, cholesterol, phospholipids and bile acids.[15,16] The pathophysiology of ChGS formation involves three steps: supersaturation, crystallisation and growth. Hypersecretion of cholesterol, cholesterol supersaturation of bile,[17] hypomotility dysfunction of the gallbladder and the accumulation of mucin gel contribute to the formation of ChGS5 (Figure 2). Cholesterol nucleation is known to be an initial stage in the formation of ChGS.[18,19] Under certain conditions, cholesterol can aggregate and precipitate in them as cholesterol monohydrate crystals to give rise to the core of a ChGS.[20]

Bile proteins[19] and bilirubin, in addition to cholesterol crystals, can be a matrix in ChGS formation. Mucin-glycoprotein gel is one of the most important and identified pronucleators. Chronic inflammation of the gallbladder wall and hypersecretion of mucin are considered important factors in the pathogenesis of ChGS.[21,22] Cystic bile destabilised by chronic inflammation of gallbladder wall contains high arachidonyl-lecithin levels (Figure 2). The observed increase in the activity of the phospholipase A2 secreted by bacteria leads to the hydrolysis of phospholipids and the accumulation of free fatty acids, including arachidonic acid.[23] The latter activates the generation of prostaglandins, thromboxanes and leukotrienes to cause mucin glycoproteins to be hypersecreted by the gallbladder mucosa. In infection, cholic acid is converted to lithocholic acid. The higher production of lithocholic acid in the cystic bile promotes aggregation of cholesterol monohydrate crystals. There are morphological changes

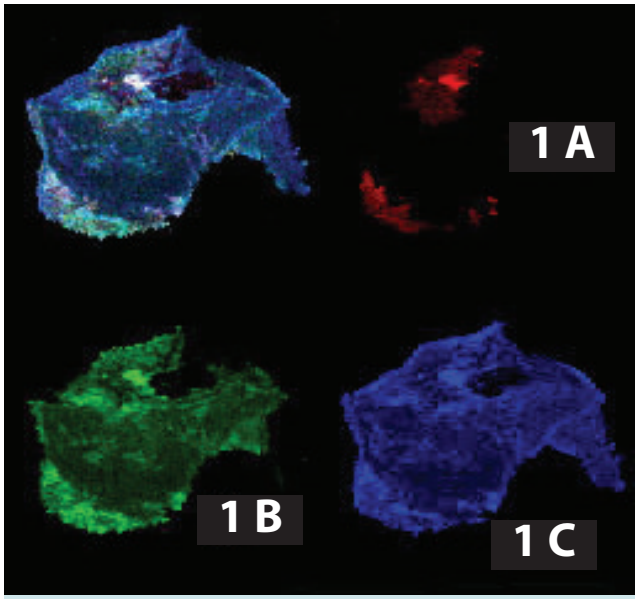

Figure 1. Micro images of cholesterol gallstones by color cathodoluminescence scanning electron microscopy. The application of the computer program "Adobe Photoshop" (software) and color contrast by the color cathodoluminescence scanning electron microscopy (CCLSEM) technique permitted the determination of cholesterol, bilirubin and protein within the stone. CCLSEM micro¬graphs of cholesterol (A), protein (B), bilirubin (C) were obtained after color separation. 11 The major components of the gallstones under examination were cholesterol (A) and protein (B). They were detected all over the entire surface of the scanned gallstone while rare bilirubin insertions (C) were seen only at the periphery of the gallstone.

in the gallbladder mucosa. The columnar epithelium flattens and microvilli are lost. This results in impaired water and electrolyte absorption processes. Cholesterol monohydrate crystals, mucus glycoprotein mucin bands and calcium bilirubinate granules form the basis for biliary sludge.

Oestrogens, prednisolone,[9] cyclosporine, azathioprine, sandostatin,[24] clofibrate, nicotinic acid and a number of other long-term medicines increase the risk for GD.[25,26] Oral contraceptives increase the incidence of GD in younger women, especially in the early period of their use of oral contraceptives.[27] Hormone replacement therapy in postmenopausal women has also been described to be associated with an increased risk for gallstone disease.

Long-term therapy with each of these agents enhances cholesterol excretion into bile and results in its supersaturation with cholesterol through competitive inhibition of bile acid synthesis from cholesterol on cytochrome P450 (Figure 3).[12] Concomitant inflammatory diseases of the gallbladder and its motor dysfunction may lead to the development of gallstone disease.

Regression and discrimination analyses reveal a strong association of gallstone formation in SLE patients with age at the onset of the disease, current steroid dosage, and duration of prednisolone therapy (Table 1).[12]

The available facts allow discussion about drug-induced gallstone disease.[9,12] The presented above data, suggest that long-term the use of medicines containing steroid hormones, cytostatics, may be regarded as a model system of development of cholelithiasis in man. Further multicentre studies are needed to prove the relationship of a GS as a result of long-term treatment of medications, metabolising on cytochrome P450. Such studies might provide clues to understanding of these gallstones' formation and could ultimately result in the development of new strategies for prevented and treatment of gallstones with these patients.

Treatment of Cholelithiasis

Surgery has long remained the exclusive form of therapy for GD.[28] Advances in the study of the physiology of bile formation[29] and pathogenesis of GD has allowed extension of indications for therapeutic treatment cholelithiasis. Surgical and medical treatments for cholelithiasis are equally used today. The main methods of treatment of GD are:

- Cavitary and endoscopic cholecystectomy
- Litholytic therapy (LT)
- Extracorporeal shock wave lithotripsy (ESWL)
- Extracorporeal shock wave lithotripsy + Litholytic therapy
- Percutaneous transhepatic LT

The final choice of treatment policy must be eventually determined by a joint decision between a therapist, surgeon and patient. This review will outline the basic principles of medical therapy for cholelithiasis.

Litholytic Therapy

GS dissolution is based on the physiology of bile formation[29] and the pathophysiology of cholelithiasis. Scientists experimentally established that a change in the ratio between the concentration of bile acids,

Variable	The Fisher Exact Test	P-value
Age at the onset of the disease	93.8	<.000001
Current steroid dosage	109.2	<.0000001
Steroid therapy duration	152.0	<.0000001

Table 1. The predisposing factors to gallstone formation in the study SLE patients.

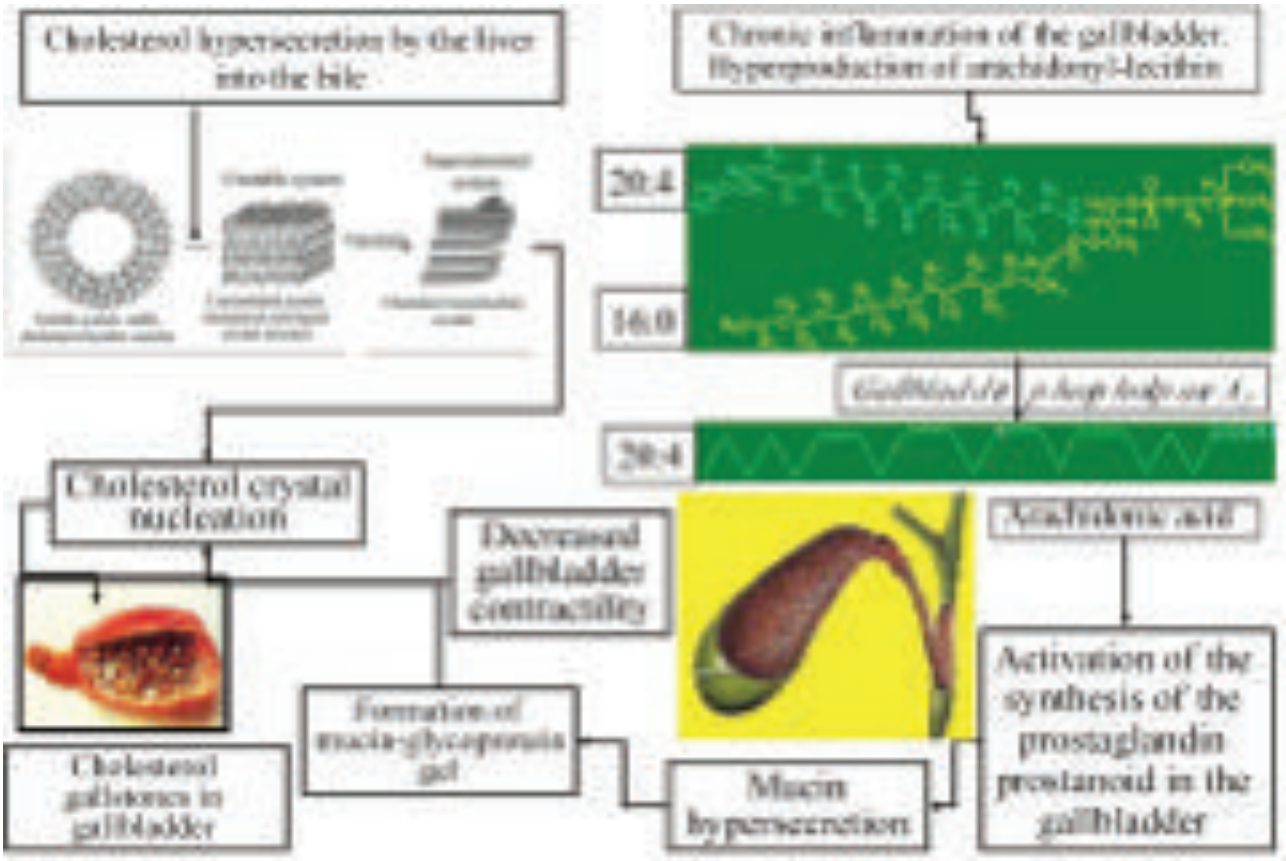

Figure 2. A diagram of gallstone formation by taking into account the above impaired bile production and excretion processes.

cholesterol and phospholipids leads to a redistribution of phases in a triangular system of coordinates (Figure 4).[30]

This principle underlies the dissolution of ChGS by using ursodeoxycholic acid (UDCA) (Figure 5). Result of application UDCA is increase of bile acids concentration in bile, inhibition of hepatic synthesis of cholesterol, decrease content of cholesterol in bile and reduction of absorption of cholesterol from the intestine, that ultimately leads to dissolution of gallstones.[28]

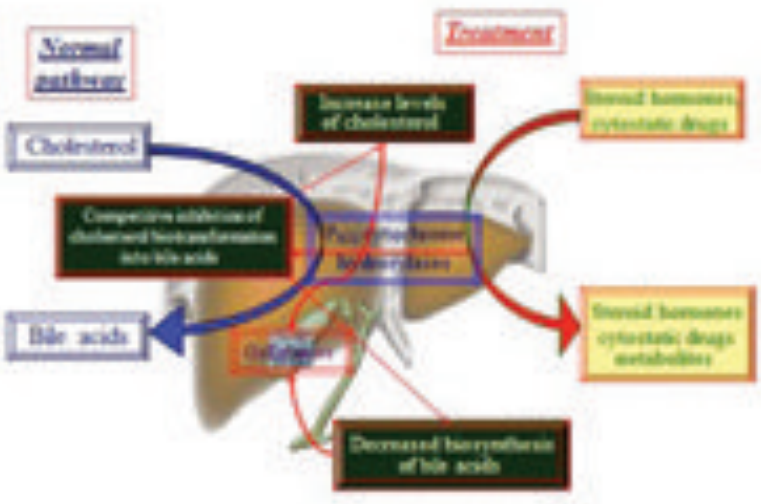

Figure 3. Diagram of steroid hormones-induced inhibition of cholesterol catabolism.

The dose of a drug depends on body weight.[10] For the highest therapeutic effect, the drug should be taken in a single daily dose overnight, for its highest concentration in the gallbladder at a relative functional rest and during the maximum cholesterol synthesis.[31]

The efficiency of litholytic therapy (LT) is shown to depend largely on its use at the early stages of GD. Drug therapy is performed long-term (from 6 months to 2 years or more), necessarily with ultrasound guidance and biochemical blood tests carried out every three months during therapy. When selecting the patients correctly, the efficiency of litholytic therapy with UDCA is as high as 60%-90%. If there is no reduction in the sizes of gallstones within 12 months of the initiation of litholytic therapy, the latter should be stopped.[31]

Unfortunately, GS may again form after their successful dissolution. After successful oral LT, recurrent stones are annually about 10% during 5 years, more frequently during the first 2 years, and then their frequency decreases. The risk for recurrence is less in patients with a primary single stone than in those who have earlier been found to have multiple stones. For the prevention of stone recurrences, it is necessary to continue small-dose UDCA therapy.

Dissolving gallstones can not only work with bile

Criteria
The stone should be cholesterol or mixed
The size of the stones should not be greater than 1.5cm
The gallbladder should fully preserve its function and be packed with stone not more than ¼ of the fasting volume
The cystic duct and common bile duct should preserve their patency
Enterohepatic circulation of bile acids should be preserved

Table 2. Criteria of patient's selection with cholelithiasis for litholytic therapy.

acids, but also phospholipids. The solubilising properties of phosphatidylcholines are shown to be largely due to the fatty acid that is in the second position of a phospholipid molecule. The laboratory studies have demonstrated that the conjugates of bile acids and fatty acids do show a cholesterol-solubilising effect.[32] The conjugates of bile acids with arachidonic acid, arachidyl-amino-cholanoid have the best solubilising effect. UDCA and/or higher dietary fat content appear to prevent formation of gallstones.[33]

Contact Litholysis

Contact litholysis is a variant of litholytic therapy. A substance that dissolves cholesterol stones is injected just into the gallbladder or bile ducts. Only cholesterol stones are prone to dissolution; their size and number are of no fundamental importance. Methyltretbutyl ether and propionic ether are used to dissolve stones in the gallbladder and bile ducts, respectively. Dissolution occurs within 4 to 16 hours. This procedure can be the method of choice in treating GD patients at high intraoperative risk.

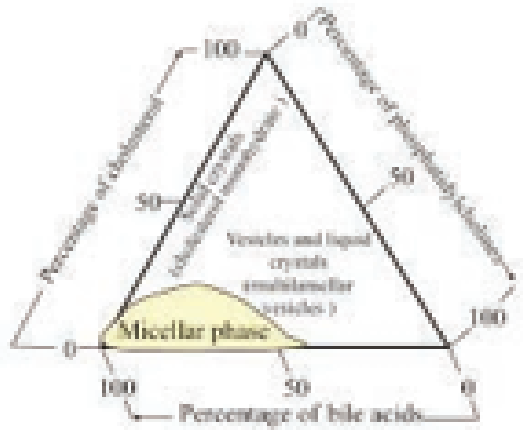

Figure 4. The phase state of the main bile components (cholesterol, phosphatidylcholines, bile acids) in the triangular coordinate system.[30]

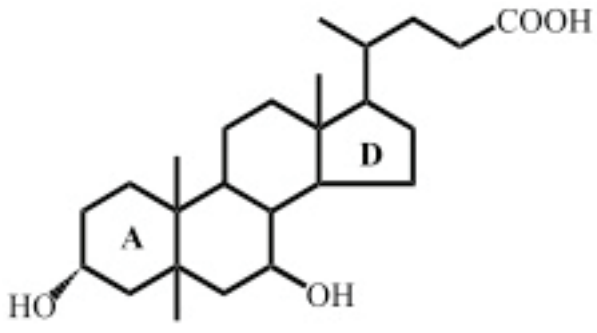

Figure 5. The structural formula of ursodeoxycholic acid.

Extracorporeal Shock Wave Lithotripsy

Extracorporeal shock wave lithotripsy (ESWL) has substantially extended the capabilities of medical treatment in patients with GD and could achieve a positive effect in those with gallstones up to 3cm in diameter. The technique is based on shock wave generation. Pressure that is 1000 times greater than the atmospheric one is achieved in the focus within 30 seconds. Because soft tissues absorb little energy, its bulk falls on a stone, causing its destruction. The technique is used as a preparatory stage for further oral litholytic therapy.

Approximately 20% of patients with GD meet the criteria for ESWL. Stone shattering into small fragments occurs after 1-3 sessions. When patients are correctly selected for ESWL, stones fragmentation can be achieved in 90-95% of cases. The most common reactions are biliary colic and, occasionally, minor signs of cholecystitis, hyperaminotransferasemia.[10] Biliary colic is eliminated by the use of spasmolytics and analgesics. After lithotripsy, stone fragments are mainly excreted independently. Shock wave lithotripsy is generally used in combination with litholytic therapy that should be continued within six months after the last session of lithotripsy. Shattering of large gallstones by a few sessions in combination with litholytic therapy prevents the development of obstructive jaundice after lithotripsy. Contact litholysis may be successfully used to dissolve fragments remaining after ESWL.[10]

High recurrence rates in the late period following lithotripsy are the most essential limitation to apply this technique.[34] ESWL has also shown to be effective in 90% of the common bile duct stones refractory to endoscopic treatment;[35] however, a recurrence is observed in 14.5% of patients within 10 years.36 There are data on the relative safety and efficiency of ESWL in patients with incorporated biliary tract stones and a high surgical risk.[37,38]

Criteria of patients selection for ESWL	Single radiolucent cholesterol stones not more than 3cm in diameter
	Multiple radiolucent stones (not more than 3) 1-1.5cm in diameter
	The volume of stones is < 1/2 of that of the gallbladder
	A functioning gallbladder
	Normal bile duct patency
Contraindications to ESWL	The presence of coagulopathy or anticoagulant therapy
	The presence of cavitary mass along the course of a shock wave

Table 3. There are strict indications for extracorporeal shock wave lithotripsy.

Factors
To avoid inactivity.46 Patients with GD are recommended to exercise (graduated walking of at least 1km daily; daily exercises associated with the tension of prelum abdominal and the elevation of intraabdominal pressure)
To keep a dietary pattern (frequent, fractional) and low-cholesterol diet
To eliminate being overweight
To avoid long-term starvation periods and intake of cholesterol synthesis-increasing drugs10
To have gallbladder ultrasonography at least once a year

Table 4. Factors are contributing to the prevention of relapses of gallstone disease.

Potential GD-preventing Drugs

Among the GD-preventing drugs, ezetimibe is noteworthy.[39,40] This agent prevents the formation of cholesterol stones in mice by reducing cholesterol absorption and bile cholesterol saturation index, intensifying bile flow and enhancing the secretion of bile salts phospholipids and glutathione, which is associated with the slightly increased expression of bile acid carriers. The major effect of ezetimibe in humans is to lower cholesterol absorption.[28,41] The drug is also effective in resorbing cholesterol stones by producing excess unsaturated micelles.[42] Ezetimibe, by acting at different levels of cholesterol homeostasis, might represent novel therapeutic approaches to prevent cholesterol gallstones.[40]

The long-term use of magnesium preparations has been demonstrated to prevent the occurrence of clinical forms of GD. Magnesium deficiency may cause dyslipidemia and insulin hypersecretion.[43,44]

There is evidence for the administration of melatonin for the prevention of GD. Melatonin is considered to lower bile cholesterol by reducing the rate of its absorption by the intestinal epithelium and by increasing the rate of its conversion to bile acids.[45]

The efficiency of litholytic therapy is shown to depend largely on its use at the early stages of GD.

In conclusion, the achievement in the study of the physiology of bile formation[47] and the pathogenesis of gallstone disease has allowed expanding indications for therapeutic treatment of GD and reducing the number of patients who undergo surgical treatment. The development of novel, effective and noninvasive therapies is crucial for reducing the costs of healthcare associated with gallstone disease. Treatment of gallstone disease, taking into account the molecular mechanisms and combined therapy will allow not only treatment but also prevent the very common worldwide disease.

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The Gut Microbiota and the Liver: Exploring the Microbiota-Gut-Liver Axis

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By virtue of its anatomical location, the unique nature of its blood supply and its critical metabolic and immunologic functions, the liver is strategically positioned to confront and interact with those microbes, microbial components and products of microbe-gut interactions that may have traversed the gut barrier and gained access to the blood stream.[1] Conversely, liver disease or shunting of portal blood so that its bypasses the liver will have serious consequences.

The Gut Microbiota

Thanks to the rapid pace of development of microbiological technologies[2] the microbiome has emerged as one of the "hottest" areas in biomedical research and much has already been learnt of the critical role of our commensal flora in health.[3,4] Most relevant to hepatology have been revelations relating to the important metabolic functions of the microbiome in health and the potential for changes in enteric bacterial populations to contribute to the pathogenesis of obesity and the metabolic syndrome.[5-7] Hepatologists will be long familiar, through their clinical experience with hepatic encephalopathy, with the concept of a microbiome-gut-brain axis.[8] This same clinical experience will also have informed hepatologists on the capacity for diet and antibiotic therapy to modulate the microbiota. In both the short and long term, diet is now recognised as a major regulator of the microbiome[9-12] and evidence accumulates to indicate that the impact of antibiotics may be more long-lived than was previously thought[13-15] and that antibiotic exposure in early life, at a time that the microbiota is being established, may have long-term consequences in terms of predisposition to certain diseases.[16]

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The Gut Microbiome and the Liver

That the gut microbiota is relevant to the natural history of liver disease was recognised over 60 years ago when relationships between gut bacteria, their metabolic products and hepatic coma were first described.[17-19] In these studies the importance of coliforms was emphasised and these same bacteria and the inflammatory response that they evoke have since been have been incriminated in the pathophysiology of portal hypertension as well as in such infectious complications of chronic liver disease as spontaneous bacterial peritonitis, systemic sepsis and hemostatic failure.[20,21]

While the role of gut bacteria in the aforementioned complications of liver disease is now widely appreciated, more recently research efforts have begun to focus on the possibility that the gut microbiota may be fundamental to the pathogenesis of various liver diseases. Indeed, evidence accumulates to support a role for the microbiota in alcoholic liver disease,[22] non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH),[5] TPN/intestinal failure-associated liver disease (IFALD)[23] and primary sclerosing cholangitis (PSC).[24,25]

In liver disease, an overgrowth of Gram-negatives allied to impaired gut barrier function allows whole organisms, through the process referred to as translocation, and/or the Gram negative bacterial component lipopolysaccharide (LPS) to gain access to the portal system. If translocation occurs, bacterial components such as LPS arriving in the portal system activate an inflammatory cascade, which ultimately leads to liver injury.[26] In exploring how the microbiota may be incriminated in the pathogenesis of liver disease three phenomena deserve special attention: small intestinal bacterial overgrowth (SIBO), quantitative or qualitative changes in the microbiota and the immunological response of the host to the microbiota.

Small Intestinal Bacterial Overgrowth (SIBO)

SIBO has been repeatedly shown to be common across a spectrum of chronic liver diseases and its occurrence has been shown to correlate with both the severity of liver disease and the likelihood of such

complications as encephalopathy and SBP.[27] That SIBO could lead to liver injury was amply demonstrated by the unfortunate experience with the development of NASH among subjects who had undergone jejuno-ileal bypass procedures for obesity.[28] Precisely how bacteria might harm the liver has been revealed, for example, by the demonstration that components of the microbiota can metabolise alcohol to acetaldehyde[29] and that certain modifications of the microbiota have been linked with both the metabolic and inflammatory components of NAFLD.[5,30]

Quantitative or Qualitative Changes in the Microbiota

While several studies have examined, using modern sequencing technologies, the composition of the enteric microbiota in liver disease, it must be emphasised that most of these studies have examined faecal samples and that bacterial populations in faecal samples may be quite different from those closely adherent to the mucosae of the colon and small intestine.[31] These methodological limitations notwithstanding, several studies have reported reasonably consistent changes in the faecal microbiome in chronic liver disease and have linked these changes to the severity and natural history of liver disease; in general, such changes involve a proliferation of members of the family Enterobacteriaceae, and a reduction in important commensals, such as Bifidobacteria spp.[32-36]

More subtle shifts in the metabolic activity of the microbiota may well be involved in the pathogenesis of one of the most common liver diseases worldwide, NAFLD. Firstly, distinctive microbial signatures have been linked to obesity as well as type 2 diabetes and the metabolic syndrome.[37-39] Secondly, changes in the microbiota have also been demonstrated in both animal models of, and human subjects with, NAFLD and NASH and evidence accumulates to incriminate a pathway linking the microbiota with toll-like receptors (TLRs) (and TLR4, in particular) and the activation of pro-inflammatory cytokines, such as TNFα.[40-45]

Immunological Reactions to the Microbiota

Though less studied, a reasonable body of evidence suggests that an immunological response to the microbiome (whether normal or aberrant) may play a role in the pathogenesis of both primary sclerosing cholangitis (PSC) and primary biliary cirrhosis (PBC). In both PSC and PBC, expression of TLR4, the ligand for LPS is upregulated and cross-reactivity between microbial antigens and human tissue components has also been demonstrated.[6,46-49]

Therapeutic implications

Antibiotics

The role of antibiotics in the management of SBP, bacteremia and hepatic encephalopathy has been established for decades.[51-54]

By removing organisms involved in the generation of endotoxin which, in turn, is thought to sustain the hyperdynamic circulation that sustains portal hypertension, antibiotics may not only prevent related infectious complications, but also reduce re-bleeding rates from variceal haemorrhage.[55,56] How precisely antibiotic, such as the poorly absorbed molecule, rifaximin, mediates its benefits in hepatic encephalopathy is unclear. The prevention or resolution of encephalopathy with rifaximin may owe more to subtle changes in bacterial metabolism than to significant changes in microbial abundance.[57,58] Apart from the aforementioned example of jejuno-ileal bypass-related liver disease there is, as yet, no convincing evidence for a beneficial effect of antibiotics in the evolution or progression of a given liver disease.

Probiotics

Despite a considerable volume of experimental data indicating the ability of various probiotic strains or strain combinations to either prevent or ameliorate the progression of liver disease in various animal models[59-61] and an ever-increasing interest in the potential benefits of probiotics, in general, the literature on the clinical impact of probiotics in liver disease and its complications is scant at present. To date, studies of probiotics in liver disease have been generally small in size and their results far from conclusive. Whether safety concerns, namely, the potential for orally administered organisms to access the systemic circulation directly in those with porta-systemic shunting is not clear. Though some positive shifts in biochemical parameters have been reported there is, as yet, no good evidence to support the use of probiotics in changes NAFLD, NASH or alcoholic liver disease.[62-65] With regard to the impact of probiotics in the management of complications of chronic liver disease, such as encephalopathy and SBP, here again the literature is limited and generally inconclusive.[66,67] However, some very recent studies indicate that in minimal HE prebiotics, probiotics and synbiotics may be effective.[67-69] Studies looking at the impact of probiotics on the natural history of chronic liver disease in general or on specific entities, such as PSC, have been very few and, to date, negative.

Summary

The importance of the microbiome-gut-liver axis has long been recognised clinically and is now being explored and its complexities revealed experimentally. In coming years, studies of the microbiome in human disease will rapidly move from being purely descriptive to being mechanistic and will reveal what bacteria and/or bacterial-derived molecules actually do and how the microbiota can be optimally manipulated. Only then will the promise of interventions such as probiotics[70] and faecal transplantation[71] be realised and precise microbial manipulations employed in the prevention and treatment of liver disease and its complications.

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Changing Prevalence of Hepatitis A Antibodies in Developing Countries

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Introduction

Hepatitis A, the most common form of acute viral liver disease worldwide, is caused by hepatitis A virus (HAV). HAV is a non-enveloped, single-stranded RNA virus, classified in the genus Hepatovirus within the Picornaviridae family.[1]

HAV is very stable in the environment, and is transmitted primarily via the faecal-oral route either through ingestion of contaminated food and water or through direct contact with an infectious person. The virus has a special tropism for liver cells producing a self-limited disease that does not result in chronic infection. The clinical manifestation includes non specific symptoms similar to those caused by other viruses, therefore serological testing of IgM antibodies against HAV (anti-HAV) is required to establish the etiological diagnosis of acute hepatitis A. The clinical outcome is strongly related to age: young children usually have asymptomatic infection, while older children and adults commonly experience symptomatic disease. Although rare, HAV infection can cause acute liver failure and death and this risk increases with age and the presence of chronic liver disease.[2]

The incidence of hepatitis A is strongly related with socioeconomic status; with increasing incomes and access to clean water and adequate sanitation, the incidence of HAV infection decreases.[2-4]

Global Epidemiology of Hepatitis A

An estimated 1.4 million cases of hepatitis A occur every year in the world.[5] Following acute hepatitis A infection and disease specific anti-HAV antibodies are detectable for years, probably lifelong, and HAV endemicity level for a population may be defined by the results of age-seroprevalence surveys.[6,7]

Serological prevalence profiles vary geographically: high-income regions present very low HAV endemicity levels, low-income regions high endemicity levels, and most middle-income regions have a mix of intermediate and low endemicity.[2,5,7]

In less developed countries, in Africa, parts of Asia and Central and South America, with very poor sanitary conditions and hygienic practices, most persons become infected in early childhood, when the infection is asymptomatic. Epidemics are uncommon because older children and adults are generally immune. In developing countries and some regions of developed countries, which include Eastern Europe, parts of Africa, Asia and America, with improving socio-economic and hygienic conditions, children often avoid infection that occurs mainly in adolescents and adults, and major outbreaks may occur. In developed countries such as North America, Western Europe, Australia and Japan, with good sanitary and hygienic conditions, infection rates are low. Disease predominates among specific adult risk groups, such

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as injecting-drug users, men who have sex with men, people travelling to areas of high endemicity, and in isolated populations such as closed communities.[2-9]

Prevelence of Hepatitis A: Focus on Developing Countries

Africa

Information on HAV infection in Africa is limited. Although the epidemiological situation of the disease varies among different socio-economic groups, available data shows that most of Africa remains a high endemicity region.[3]

Sub-Saharan Africa has some of the highest anti-HAV prevalence rates in the world and nearly all older children and adults may be considered naturally immunised. Seroprevalence studies in this area were mostly performed in the 1990s and few recent data are available. A recent survey performed in a small sample of blood donors and pregnant women in Burkina Faso, a country characterised by low income and poor hygienic status of a major part of the population, shows an anti-HAV prevalence of 14.3 and 23% respectively, lower than expected even in selected population groups.[10]

Typically, the transition from high to intermediate endemicity is detected in North Africa. Studies from the 1980s showed nearly universal immunity in many countries, with 100% anti-HAV positive by age 10 years in Algeria and in adults in Morocco. More recent data, however, shows that the urban areas and the higher social classes have experienced a decline in hepatitis A infection.[3,7]

This phenomenon was confirmed in Egypt where anti-HAV seropositivity in children aged <6 years was 27.3% in high and 81.0% in low social class.[11]

A study recently performed in South Tunisia confirms the epidemiological transition underway in North Africa and the decline of the infection following the improvement of the living conditions of the populations. In fact the seroprevalence decreased from 95% in 2000 to 86% in 2007 and the decline in hepatitis A infection was higher in the urban area compared with rural areas.[12]

The relationship between social level and the degree of infection was document in South Africa where almost all black adults (90%) were positive for anti-HAV, while only half of the white adults (40-60%) showed previous contact with the virus.[7,13]

Asia

In many rapidly developing countries of Asia, surveys of anti-HAV antibody in the population show transition from high to intermediate endemicity with areas of low prevalence. In these regions, where a substantial proportion of adolescents and adults are susceptible, HAV may circulate often through community-wide outbreaks.[2] Some recent seroprevalence data, representative for different Asian countries, are shown in Table 1.

In Korea, where the socioeconomic status showed a steady improvement accompanied by an increase in individual and social hygiene in the past 30 years, the susceptible population changed from subjects under 10 years of age to subjects aged 10-29 years. Many surveys on anti-HAV prevalence were performed and all confirmed the changes from high to intermediate endemic area.[14-17] In 2008-10, the seroprevalence was 53.8% and, when analysed according to age, gradually increased from children to adults.[16] Similar data were obtained in a nationwide study considering over 60,000 samples which showed no significant difference according to sex and region.[14]

In China, a cross sectional study performed in six areas showed an overall prevalence of anti-HAV of 72.9%, with an increase from younger to older groups.[18] The change in seroprevalence is the result of the decrease in the national incidence rate of acute hepatitis A observed in the last decades. This decline was made possible by the rapid urbanisation and improved sanitation of the Chinese populations and by the implementation of the program of HA vaccination for children.[19]

Though incidence is low, due to declining prevalence of immune subjects, large outbreaks may occur resulting in huge economic loss. Following the large epidemic involving about 310,000 people in Shanghai in 1988, other outbreaks occurred and recently several cases of viral hepatitis A were reported to the Guangxi CDC from a middle school in a rural location, likely caused by contaminated well water.[20]

The anti-HAV seroprevalence in Taiwanese adolescents dramatically decreased from 89% in 1975 to 2.3% in 2011. In Taiwan the economic development starting in the 1970s, accompanied with better living standard, education, and hygiene, played crucial roles for the decline of hepatitis A incidence.[21] For example, in 1982 the government implemented a policy of nationwide disposable tableware use in public eating places. Data from some studies in different time periods supported the effectiveness of the intervention in preventing the transmission of hepatitis A.[22]

In Taiwan, HAV prevalence is decreasing also in adults reaching levels similar to those detected in Japan, a low endemicity area where the prevalence has decreased markedly in the last years.[23] Therefore, in these populations new risk groups for infection are identified that can be targeted for immunisation campaign.[24]

India showed a very rapid socio-economic development in the last years; many high endemicity areas for HAV infection coexist with others making a transition to moderate incidence. Anti-HAV positivity varied from 26% to 85%; in particular, almost 50% of children between the ages 1–5 years were found to be susceptible to HAV.[25-27]

In Iran, an area where HAV infection is still highly endemic, the overall seroprevalence of anti-HAV in the general population of three provinces was 86% and the data are confirmed in one of the largest provinces in the South where anti-HAV prevalence was 88%.[28,29]

However, in an area where an increase of socioeconomic and sanitary status was present, a decline of hepatitis A infection and anti-HAV levels in the community was detected and the seroprevalence in children aged 1-15 was 3.6%.[30]

Central and South America

Improvements in public health programs and sanitary conditions had a strong impact on the epidemiological patterns of HAV infection in several Latin American countries where the seroprevalence is changing from high to intermediate endemicity (Table 2). In contrast, the Andean regions have the highest prevalence of HAV in the Americas (94%). No national trend or shift in the epidemiological pattern was seen in Bolivia, where the overall prevalence of anti-HAV antibodies in children was 95.4%. However, some changes are probably occurring as the prevalence of anti-HAV was significantly higher in children with parents who had a low education level.[31]

In Brazil, available studies indicate different patterns of endemicity. In São Luís, a town in the North of the country, anti-HAV was positive in 64% of elementary schoolchildren and prevalence reaches up to 95% in the North-Northeast and Central-West regions.[32,33]

In the South and Southeast regions prevalence tends to be lower and in Santos, a town in the South, only 9.7% of children and teenagers were anti-HAV positive.[33,34]

The differences in seroprevalences among regions are probably the result of the socioeconomic and hygienic conditions of these areas. This transition of HAV epidemiological pattern from a high to a medium endemicity is important to public health as the delay in the age of exposure may increase the burden of disease in adolescents and young adults, while older adults are generally immune with a seroprevalence rate reaching 100%.[35,36]

Also in Mexico, where the HAV endemicity is intermediate, adolescents

Country	Year	Age	Socioeconomic level	HAV+ %	References
Korea	2008-2010	15-19 years	general population	13	16
Korea	2008-2010	30-39 years	general population	52.2	16
Korea	2008-2010	70-79 years	general population	95	16
China	2007	6-10 years	general population	51.88	18
China	2007	≥60 years	general population	97.7	18
Taiwan	2011	7-15 years	school children	2.3	21
Taiwan	2011	adults	teachers	52	21
India	2012	1-5 years	general population	≈50	27
India	2010	>16 years	general population	80.8	7
Iran	2008-2009	<20 years	general population	79.3	29
Iran	2008-2009	>30 years	general population	99	29

Table 1. Seroprevalence of Hepatitis A in Asia. 2008-2012.

Country	Year	Age	Socioeconomic level	HAV+ %	References
Brazil MW*-SE*	2007-2009	1-4 years	low	10-13.2	33
Brazil MW*-SE*	2007-2009	15-18 years	low	57.4-68.9	33
Brazil NO*	2007-2009	1-4 years	low	25.9	33
Brazil NO*	2007-2009	15-18 years	low	80	33
Mexico	2010	1-9 years	general population	45	37
Mexico	2010	10-19 years	general population	80	37
Mexico	2010	≥20 years	general population	96.9	37
Bolivia	2010	5-16 years	school children	95.4	31
Argentina	2009-2010	15-25 years	low	62.3	38
Argentina	2009-2010	15-25 years	high	41.5	38
Argentina	2009-2010	>56 years	general population	98.7	38

Table 2. Seroprevalence of Hepatitis A in Central and South America. 2007-2010. *MW Midwest, SE Southeast, NO North

represent a group at high risk of infection.[37]

In the past, Argentina was considered to be an area of high endemicity for HAV infection, with most people infected in early childhood. In 2005, vaccination against HAV was included in the national immunisation program for children aged 12 months. Concomitantly, the mean age of HAV infection decreased and different prevalence patterns related to socioeconomic level were observed. In Córdoba City prevalence of anti-HAV was detected in 81.9% in low-income populations and 66.2% in middle/high-income groups. Environmental surveys allowed the detection of hepatitis A wild virus and, due to the decrease of immune subjects, the possibility of outbreak is increasing.[38]

A shift in prevalence is presently also observed in Chile where HAV infection decreases as sanitary conditions improve along with the socioeconomic situation.[39]

Discussion

The incidence of HAV infection and the prevalence of antibodies against HAV are closely associated with economic development and access to safe drinking water and sanitation. As individual income increases the incidence of HAV infection decreases; therefore, anti-HAV prevalence is an indicator of the development of the world areas. Serological prevalence is strictly related with the socioeconomic situation. In the last decades, a dramatic change in the epidemiology of HAV infection happened in developed countries, where adults also have no markers of previous infection. The same situation is now characterising many developing countries where a shift in the age of primary infection is detected. In poorest countries, hepatitis A is still a benign children infection. In developing countries the rapid decrease of asymptomatic children infections and the presence of susceptible older groups is the base for hepatitis A burden of disease. Care and societal costs represent a significant public health problem as outbreaks may occur and risk groups are often identified.

Therefore in developing countries a comprehensive plan for the prevention and control of viral hepatitis A should also be adopted, including measures to improve hygiene and sanitation and a specific vaccination strategy with available safe and effective vaccines. In high endemicity settings, the World Health Organization (WHO) does not recommend large-scale vaccination as almost all persons are asymptomatically infected with HAV during childhood and in low endemicity settings, vaccination is recommended only for individuals with increased risk of infection. In intermediate endemicity settings, where seroprevalence changes rapidly occur, the WHO recommends that large-scale childhood vaccination may be considered as a supplement to health education and improved sanitation, on the basis of the incidence of acute hepatitis A and of cost-effectiveness analysis.[2]

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How to Optimise the Prevention of HBV Vertical Transmission?

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Introduction

Hepatitis B virus (HBV) infection is one of the most common viral infection in the world, with more than 350 million chronically infected people.[1] This burden is responsible for about 1 millions deaths per year, largely linked to hepatocellular carcinoma (HCC),[1] despite the availability of an extremely effective vaccine for over 25 years.[2] This is closely due to insufficient public health policies in high endemic areas (Asia and Africa) where more than 8% of the population is chronically infected, and where the implementation of an efficient preventive strategy is difficult. The persistence of chronic infection in these areas is largely due to vertical (mother-to-child) transmission.

Vertical HBV Transmission

In high endemic regions, most infections occur at birth or at a very young age. These infections remain asymptomatic and the risk of chronic evolution is very high. In South-East Asia, in the East or in Pacific regions, 30 – 50% of chronic infections are of vertical origin linked to a high viral load in mothers.[1, 3, 4] In Africa, South-America and in the Middle East, where viral loads seem to be less, vertical transmission represents only 10 – 20% of chronic infections.[1, 3, 4]

Vertical HBV transmission occurs essentially during delivery. In mothers with high HBV replication rate detected by hybridisation (equivalent cut-off at 700,000 copies/mL), the risk of vertical transmission is about 90% in the absence of prophylaxis and the risk of chronicity for the newborn is about 80 – 90%.[5, 6] Maternal infection by a pre-C mutant (HBeAg negative mothers), which is associated with a lower viral load than the wild-type virus (HBeAg positive mothers), reduces the risk of transmission and of chronic infection in the newborn. In absence of detectable maternal HBV-DNA, the risk of infection is between 10 – 30% depending on the cut-off level of HBV-DNA testing.[5, 6]

Usually, vertical transmission is due to chronic maternal infection. However, if an acute HBV infection occurs in the 3rd trimester of pregnancy, the active viral replication at time of delivery explains that it is transmitted in 65 – 100% of cases.[7, 8] Due to the long incubation time of HBV (6 weeks to 6 months), acute hepatitis in the post-partum period can also cause infection, which is likely attributable to the maternal viral load at time of delivery in absence of any clinical manifestations.

Recommendations for Vaccination and Sero-vaccination

World Health Organization (WHO) recommended that routine infant immunisation programmes were implemented in all countries.[9] Furthermore, in high endemic countries, WHO recommended that the first dose of hepatitis B vaccine was given as soon as possible and in practice before 24 hours of life, corresponding to a birth-dose vaccine without testing the HBsAg status in mothers.[9] In 2006, 163 of 193 WHO members (84%) have introduced hepatitis B vaccination in their national infant immunisation programme and 81(42%) birth-dose vaccine.[10] For the sub-group of high endemic countries, the implementation rates were 84% and 44%, respectively.[10] However, the implementation of a birth-dose vaccine could be

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challenged in resource-limiting countries for economical and logistical reasons, especially for home deliveries.[11]

In developed countries, like US or Western Europe where Hepatitis B Immune Globulin (HBIG) are disposable, it is usually recommended to test the maternal HBsAg status and if the mother is HBsAg positive, to administrate sero-vaccination to the newborn in the first 12 hours of life, which consists of a first IM injection of HBV vaccine, and at another site, an IM injection of HBIG;[7] booster vaccine injections have to be done at 1 and 6 months for completing vaccination. The vaccination schedule of 0, 1 and 6 months with paediatric dose (10 µg) is usually used except in some countries for premature birth with a 0, 1, 2 and 12 months schedule. The dose of HBIG is usually 100 IU (or 30 IU/kg). In some countries also, in case of maternal HBeAg positivity (i.e. at risk of high viral load), a dose of 200 IU at birth, repeated at 1 month was proposed, sometimes associated with a double dose vaccination. Despite its frequent use, there is no clear demonstration of the superiority of this reinforced sero-vaccination schedule. In some developing countries, this strategy of screening and sero-vaccination is also possible despite economical and logistical limitations.[12, 13]

Effectiveness of Sero-vaccination

A lot of trials have compared vaccination alone, HBIG alone and sero-vaccination with different schedules with placebo or no intervention.[7] Briefly, vaccination alone or HBIG alone is better than placebo or no intervention, but sero-vaccination is better than vaccination alone or HBIG alone.[7] Lee *et al*. did not find any difference between doses, schedules, time of the first injection (< 12 hr, 12 to 24 hr or > 24 hr) and plasma derived or recombinant vaccine for the efficacy of prevention of vertical transmission.[7] However, a lack of power due to small number of newborns in sub-group analysis could be advocated. Furthermore, these trials usually did not stratify on the maternal viral load but only in some cases on the HBeAg status.

Sero-vaccination prevents mother-to-child vertical HBV transmission in 89 to 100% of cases: in 85-92% of newborns of mothers with active viral replication and in 100% of newborns of mothers with non-replicative HBV.[7, 8] Children will develop an adequate anti-HBs response (which is supposed to be evaluated within the first year of life) with a protective titer over 10 mIU/mL in 80% of cases. Then, antibody concentrations decrease over time in such a way that around 80% of these children will maintain effective protection 5-14 years later. Recently, a large study performed in 640 children who completed sero-vaccination demonstrated the persistence of protective anti-HBs antibodies in 70, 40 and 25% of children at 5, 10 and 15 years of age.[14] The question of whether sero-vaccinated children should be called back for booster vaccination after the age of 15 years remains a matter of debate, but is not a recommendation.

Systematic sero-vaccination - or at least vaccination- of children born from HBV infected mothers, followed by of all children and adolescents then by universal vaccination, has been performed in Singapore, Taiwan and Alaska: such a policy yielded a major reduction in the rate of HBsAg carriage, a significant reduction in acute and chronic hepatitis B and in liver-related mortality, mostly by reduction of HCC rate both in adults and in children.[15-17] The neonatal HBV vaccination was the first to demonstrate that a vaccine was able to dramatically reduce the risk of cancer.

Sero-vaccination Failures

Sero-vaccination is a very potent but not totally efficient method to prevent vertical HBV transmission. Failure to sero-vaccination could be related to the lack of adherence to recommendations which is probably the main problem in western countries, and have to be regularly evaluated and corrected. Variations in the S gene have been also associated with vaccination failure, but probably plays a minor role in clinical practice.

Finally, *in utero* HBV transmission is probably the main cause of sero-vaccination failure especially in Asia. There is now great evidence in the literature that high maternal HBV DNA levels are associated with a risk of HBV vertical transmission despite a complete sero-vaccination.[18-21] This probably reflects an in utero transmission as suggested by the positivity of HBV markers in peripheral blood in infants at delivery.[18] Other factors, like HBeAg maternal status or mode of delivery, do not seem to be associated with *in utero* transmission when maternal HBV DNA is systematically measured. The highest HBV DNA levels are found in HBeAg positive pregnant women especially during the immune tolerance phase, and this could explain the role of maternal HBeAg status previously advocated in older studies.

If the correlation between maternal HBV DNA level and sero-vaccination failure[18-21] is clear, the cut-off "at risk" level is not clearly determined: sero-vaccination failure occurred at a level as low as 5 log IU/mL but the risk is significant only above 7 to 8 log IU/mL.[21]

Anti-HBV Analogues in Late Pregnancy to Reduce *in utero* HBV Transmission?

The proof of concept for a benefit of the administration of an anti-HBV analogue in late pregnancy associated with sero-vaccination on the risk of HBV vertical transmission is now clearly demonstrated. A recent meta-analysis, including 15 randomised control trials, demonstrated a clear benefit of the addition of lamivudine to sero-vaccination on both *in utero* HBV transmission, evaluated by HBsAg positivity (Relative Risk around 0.33 to 0.43) or HBV DNA positivity (Relative Risk around 0.33) at birth or at 6 to 12 months of life.[22] Moreover, this meta-analysis suggested that the benefit for late administration on lamivudine in pregnancy was only effective if the maternal viral load decreased under treatment below 6 log at delivery.[22] Similar results have been recently reported with Telbivudine and there is no benefit to introduce the pre-emptive treatment at the second instead of the third trimester of pregnancy.[23, 24] Finally, if the treatment was not

indicated for maternal liver disease, the anti-HBV analogue could be stopped between 1 to 3 months after delivery without significant increase in the risk of ALT flares.[25]

Among anti-HBV analogues, some safety data in human pregnancy were disposable only for lamivudine, telbivudine and tenofovir.[26] Telbivudine and tenofovir are listed by the FDA in the B category for pregnancy safety, although lamivudine is classified in C. Tenofovir and lamivudine have a long resume in pregnant women since more than a quarter of HIV positive pregnant women are currently on tenofovir-including regimen. Recent data suggested also that tenofovir, contrary to lamivudine or telbuvidine, could be administrated in case of breast-feeding since very few if any is excreted in human milk and absorbed by newborns.[27] Finally, tenofovir appears to be a good alternative since this drug demonstrated a high anti-HBV activity with no anti-HBV resistance to date. Even if the safety profile of these drugs is encouraging, more data have to be collected on long-term safety among children *in utero* exposed to these analogues.

Conclusions

Universal HBV vaccination strategies is effective and cost-saving in countries of intermediate or high endemicity, with less consistent benefit in countries of low endemicity. These efficient strategies are economically difficult in developing countries, but are frequently sub-optimal in developed nations in relation to the lack of systematic adherence to general recommendations. Specific strategies are also implemented in pregnant women. Systemic HBsAg detection during pregnancy followed by optimal sero-vaccination of newborns is a very efficient method to prevent HBV vertical transmission. However, it is insufficient in some cases, related to *in utero* HBV transmission associated with high maternal viral load (above 7 to 8 log IU/mL). In these particular cases, the role of anti-HBV analogues during the 3rd trimester of pregnancy, associated with sero-vaccination, as to be discussed to optimise the prevention of vertical transmission. This strategy could also decrease the need for reinforced sero-vaccination and for caesarian section, which is sometimes recommended in some countries in these cases.

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Molecular Escape Mechanisms from Treatment with NS3 Protease Inhibitors in Chronic Hepatitis C

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Introduction

Hepatitis C virus infection is an important cause for end-stage liver disease and hepatocellular carcinoma, with approximately 170 million people chronically infected worldwide.[1] Tremendous strides have been made in the development of effective antiviral drugs that can be administered orally to block viral replication, with the ultimate goal to eradicate the virus. The first two approved direct-acting antiviral agents (DAAs), telaprevir (Incivek®) and boceprevir (Victrelis®), both ketoamide inhibitors of the virus NS3 serine protease, are indicated for use in patients with genotype 1 HCV infection. They mark the beginning of an extraordinary new era in HCV therapy.[2] The high genetic diversity of HCV within infected persons, however, is a challenge for effective small molecule antiviral compounds, with clinical trials showing rapid selection of resistance-associated amino acid variants (RAVs), most of which are considered to pre-exist. Viral variant fitness and the degree of resistance are two major determinants driving their selection from within the viral quasispecies under drug pressure.

Resistance Development Against Ketoamide Protease Inhibitors

RNA viruses such as HCV generate a "cloud" of variant viruses within infected individuals. The so-called quasispecies population accumulates mutations over time due to nucleotide misincorporation by the viral NS5B polymerase and absence of proof-reading activity. Mathematical arguments suggest that every possible RAV is likely to pre-exist at a low frequency in the replicating viral quasispecies of the typical HCV-infected patient.[3] Given this genetic diversity, it is possible that naturally occurring polymorphisms in the NS3 sequence could provide a priori resistance to protease inhibitors, and thus negatively impact the success of future treatment regimens. Up to 5% of DAA-naïve patients show baseline NS3 protease RAVs,[2] however, early evidence suggests no significant impact on virus eradication as reported from triple regimens containing telaprevir (PROVE 1 and 2 trials[4, 5]) or boceprevir (SPRINT-2 trial[6]). Thus, baseline RAVs may be of limited clinical significance at present as they are likely suppressed by the peg-interferon/ribavirin backbone in current standard-of-care regimens. However, they can be expected to be of substantial importance to future interferon-sparing, all-oral combination therapies.[7, 8]

Natural variation in residues that neighbour ketoamide compounds in the ligand-binding site of the NS3 protease has recently been analysed in genotype 1a HCV.[9] Dominant strains were retrieved from DAA-naïve patients collected from geographically diverse sites, previously deposited in the public European HCV database[10] (http://euhcvdb.ibcp.fr/euHCVdb/). Such binding-site variants in NS3 at one or more ketoamide-neighbouring residues are found in approximately 7.8% of the genotype 1a sequences analysed in this study.[9] Overall, 13 different variants in the ligand-binding site have been identified (Figure 1), from which some amino acid changes (Q41H, T42A, T42S, V55I, I132V, K136R, F154Y, and T160A) have not been identified in previous *in vivo* or *in vitro* studies. Importantly, four of these dominant variants from DAA-naïve patients, Q41H, I132V, R155K, and D168G, cause low-to-moderate levels of ketoamide resistance in HCV cell culture, from which three are highly fit (Q41H, I132V, and R155K) (Figure 2).[9]

Although boceprevir and telaprevir are both linear ketoamide compounds, they show distinct structural features which contribute

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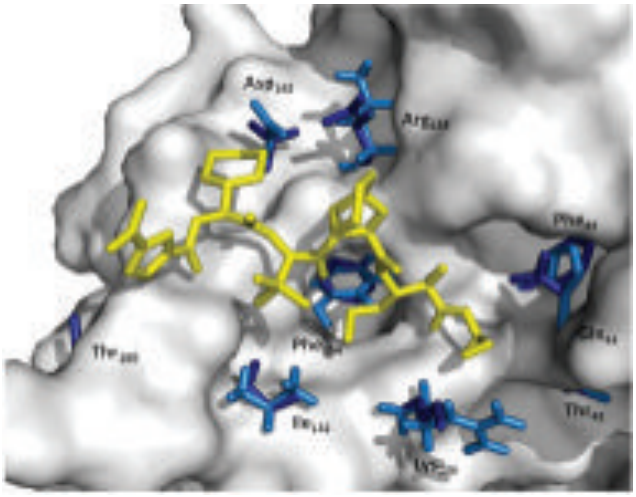

Figure 1. NS3 protease ligand-binding site variants. Surface representation of the natural substrate binding site of the NS3 protease with co-crystallised telaprevir-like ligand from Protein Databank structure 2P59 and binding-site variants identified in HCV genotype 1a dominant strains. Adapted from Welsch *et al.*[9]

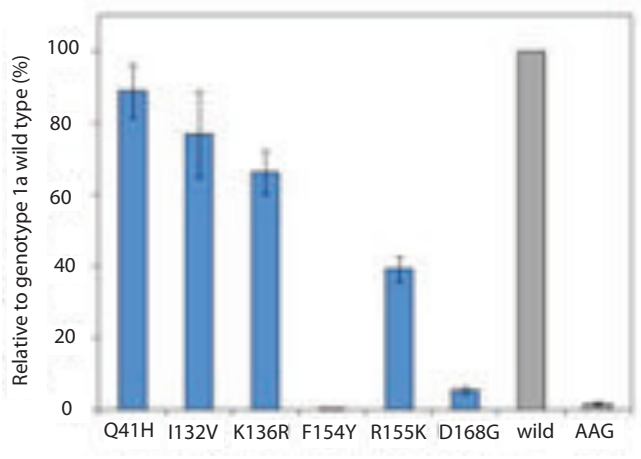

Figure 2. RNA replication fitness of NS3 protease binding-site variants. Replication capacity of NS3 protease ligand-binding site variants in H77S.3 cell culture (wild: wild type; AAG: negative control). Adapted from Welsch *et al.*[9]

to different half maximal effective concentrations (EC50) against genotype 1a HCV in cell culture and could also pose a distinct risk for RAV selection among binding-site variants. Telaprevir possesses a different P4 capping group and a cyclopropyl group at P1′ that is not present in boceprevir. Furthermore, the P2 group is different in both compounds with a smaller isopropyl-proline in boceprevir than the cyclopental-proline in telaprevir (Figure 3). Based upon *in silico* structure modeling, two ketoamide-neighboring variants that were already previously identified in clinical settings, R155K and D168G, are likely to affect binding of telaprevir more than boceprevir. Measurements of antiviral susceptibility in cell culture studies are consistent with this observation. Here, R155K and D168G led to a 2- to 4-fold greater increase in the EC50 of telaprevir compared with boceprevir, and almost a 9-fold increase in the telaprevir EC50.[9]

Varient Fitness in Ketoamide Resistance

The quasispecies comprise genetically distinct but closely related viral genomes that are competing within a highly mutagenic environment. Typically the wild type as dominant strain is detectable along with minor strains that are present at much lower frequencies, depending largely on the viral fitness of the variant strains.[11, 12] The fitness of a virus can be defined as its "relative ability to produce infectious progeny".[11, 12] Variants that harbour resistance mutations are usually less fit in RNA replication and/or infectious virus production and thus present in much smaller quantities in the quasispecies population than the wild-type virus. The replication capacity is one measure of variant fitness which depends on proper processing of the viral polyprotein by the NS3 protease. Since ketoamide compounds are mimicking the natural substrate, they likely select for binding-site variants that interfere with their binding to the protease substrate-binding site. Conversely, they can also interfere with protease substrate recognition and cleavage. Thus, binding-site variants can be expected to negatively influence RNA replication due to altered recognition of the polyprotein substrate related to structural changes similar to those leading to ketoamide resistance. Besides RNA replication, the capacity to assemble infectious virus particles contributes to the fitness phenotype of a mutant virus. Although yields of infectious virus generally correlate well with the replication capacity, some NS3 protease mutants in a cell-culture model of HCV infection reproducibly demonstrated greater impairment in their ability to produce virus than predicted from reductions in their RNA replication capacity.[13] These particular mutations nestled together at one edge of the protease substrate-binding site, which abuts the helicase domain in a crystallographic structure of the full-length NS3 molecule. It is likely that these residues are involved in domain-domain interactions between protease and helicase required for virus assembly as they did not demonstrate defects in viral egress from infected cells and no significant difference in the specific infectivity of extracellular particles.[13] Although modest in magnitude, such defects might be exponentially magnified during the multiple cycles of cell infection occurring in an infected patient. Despite this, most of the resistant mutants with a specific drop in their infectious virus yield have been identified in patients previously enrolled in clinical trials of ketoamide compounds. A possible explanation for this observation is second-site mutations with compensatory mechanisms for protein structural changes.

Compensatory Mutations, Long-term Survival and Wild-type Reversal of Ketoamide Resistant Variants

RAVs could become fixed in the viral quasispecies population due to compensatory second-site mutations. As an example, the binding-site variant F154Y is found lethal for RNA replication when placed in the background of the genotype 1a HCV strain H77S.3 in cell culture (Figure 2).[9] The complete loss of RNA replication could be due to direct interaction/clash of the Tyr154 side-chain with the polyprotein substrate. The presence of Tyr154 in the public sequence database, however, suggests that the F154Y amino acid variant is capable of functioning in an alternative sequence context, as second-site substitutions in the same strain might compensate for fitness deficits.

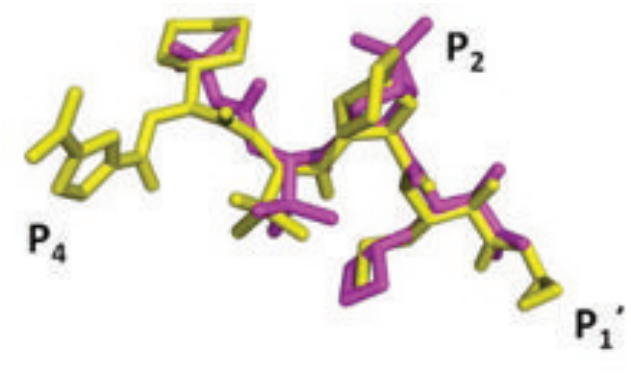

Figure 3. Scaffold of ketoamide NS3 protease inhibitors. Ketoamides boceprevir (magenta) and telaprevir-like ligand (yellow) with distinct chemical structural differences at P4, P2 and P1'. Adapted from Welsch *et al*.[9]

In fact, the respective sequence from the European HCV database containing the F154Y substitution showed two additional substitutions in NS3 that differ from the genotype 1a consensus sequence.[9] The emergence of such compensatory mutations capable of rescuing impaired replication capacity offers the potential to escape from drug pressure under DAA therapy. Their selection may allow resistant strains at a low but evolutionarily neutral and highly connected region in the overall fitness landscape to outcompete other viral variants that are located at a higher but narrower fitness peak in which the surrounding mutants are less fit. Another example is the NS3 protease RAV V55A, which has recently been identified by clonal sequencing in a long-term follow-up study in genotype 1 HCV patients who previously received telaprevir or boceprevir as monotherapy.[14] One patient showed V55A as dominant strain already at baseline, and this was still detectable at long-term follow-up. This observation is unexpected, taken into account the compromised variant fitness of this variant virus with RNA replication of only 28% compared to wild type and a distinct drop in infectious virus yield leading to a relative infectivity of only 3.1% compared to the wild-type virus (Figure 4).[15] The steep decline in infectious virus yield is particularly surprising given the fact that the V55A variant is found repeatedly in public databases. Accordingly, second-site changes may explain how this variant could become fixed in the viral quasispecies and even dominate in some treatment-naïve and -experienced patients.

Given the results from clinical trials with regimens combining multiple classes of DAAs, there is still potential to achieve viral cure even in patients who fail on a protease inhibitor-based triple therapy.[2] Without compensation for fitness deficits, RAVs that emerge during therapy clear over time, allowing the wild-type virus to re-establish and dominate. However, the appropriate waiting time before potential protease inhibitor re-exposure is unknown.

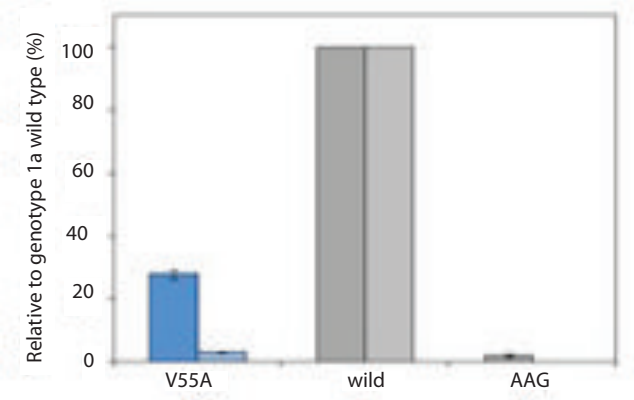

Figure 4. RNA replication and infectious virus yield of the ketoamide resistant V55A variant. Replication capacity (left) and infectious virus yield (right) of the V55A NS3 protease RAV in H77S.3 cell culture (wild: wild type; AAG: negative control). Adapted from Welsch *et al*.[15]

Conclusions and Future Perspectives

Viral fitness coupled with the degree of resistance conferred by resistant variants is likely to be the major determinant driving selection of variants from within the viral quasispecies during therapy. Most resistant variants show distinct fitness deficits from either impaired RNA replication and/or reduced infectious virus yield. However, second-site changes may explain why some resistant variants persist upon treatment discontinuation while others do not, and how resistance-associated variants that negatively impact virus replication could dominate in some treatment-naïve patients. Such naturally existing variants are found in dominant virus strains before DAA exposure. They are of substantial relevance to the success of DAA-containing regimens, but may be of limited clinical significance at present as they are likely to be suppressed by peg-interferon/ribavirin. These variants might also affect future generations of inhibitors depending upon their chemical structures. Thus knowledge on the natural variability in structures targeted by antivirals can help guide the development of future generation DAAs. The peg-interferon/ribavirin backbone in the current standard of care needs to be replaced for future all-oral regimens by another backbone with a low chance of resistance development.

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Conflict of Interest

The author has no conflict of interest to declare.

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Signaling Pathways as Therapeutic Target for HCC Treatment

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In the last decade, the incidence and mortality of hepatocellular carcinoma (HCC) have increased worldwide so that the 5-year relative survival rate for patients with HCC is still low.[1] The poor prognosis of patients with HCC is due to that most patients are diagnosed at advanced stage and the current systemic or regional chemotherapy did not show a progression. Unfortunately, the available systemic chemotherapy, such as doxorubicin-based regimens, showed only low response rate with a minimal impact on survival.[2-4] Although surgery can achieve long-term control in patients with early stage HCC, the minority of these patients are eligible for potentially curative therapies.[2] Accordingly, the improvement of the available therapeutic strategies of HCC is urgently needed. Currently, the therapeutic progression of HCC in response to the treatment with the anti-angiogenic drug sorafenib, paved the way for the clinical trials testing novel molecular targets that were thought to have therapeutic benefit for HCC patients.[5] The intensive studies on molecular targets of interest led to the expansion of angiogenesis-based therapy targeting oncogenic associated signaling pathways as a basis for the establishment of a novel therapeutic strategy for the treatment of advanced HCC. The investigation of EGFR, FGFR, PI3K/Akt/mTOR, TGF-β, c-Met, MEK, IGF signaling pathways, and histone deacetylase is based on their potency as a therapeutic target for HCC treatment.[6]

Currently, the treatment strategy of HCC is shifted from advanced stage to early or intermediate stages and from single therapy to the combination of local therapy with targeted agents.[7-9] Although this therapeutic strategy is limited to the use of novel agents following curative treatments that still remain under clinical investigation, their application in HCC treatment gained more attention in the last years.[10-12] Thus, based on the essential role of signaling pathways in tumour progression and resistance, this review focuses on the feasibility of the aberrant signaling pathways as potential target for HCC treatment. Figure 1 Outlines the signaling pathways and the corresponding moleculary targeted therapies in HCC.

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Agents Targeting EGF Signaling Pathway

After two decades of intensive research on the epidermal growth factor receptor (EGFR), now it is the most discussed potential target for cancer therapy. EGF receptor, also known as Erb1 and Her1, belongs to the ERB family of receptors tyrosine kinases. Up on ligand bindings EGFR forms either homo or heterodimers that, in turn, result in its auto-phosphorylation leading to the activation of several downstream signaling pathways, such as PI3K/Akt/mTOR and Ras/Raf/MEK/ERK pathways.[13,14] Accordingly, based on its molecular structure and activation manner, the EGF/EGFR pathway can be targeted either by the monoclonal antibody cetuximab alone or in combination with cytotoxic chemotherapy, or by small-molecule tyrosine kinase inhibitors.[15,16] Although the targeting of EGF pathway by cetuximab did not show any significant benefit for patients with advanced HCC,[17,18,19] the combination of cetuximab with chemotherapeutics, such as capecitabine plus oxalipatin as well as Gemcitabine plus Oxaliplatin showed a response rate of 20%.[20-22] Also, the reliability of EGF pathway as target for tyrosine kinase inhibitors including erlotinib, gefitinib and lapatinib, has been investigated for HCC treatment.[23,24,27] Although there is no correlation between EGFR expression and the therapy outcome, the combination of erlotinib with docataxel,[28,29] or with bevacizumab[23,30] in patients with advanced HCC showed objective responses; some patients showed disease stabilisation with variable median duration up to 68 weeks. Gefitinib is recommended as a selective inhibitor of EGFR based on its ability to inhibit growth of HCC derived cell lines.[31,32] However, the evaluation of the therapeutic reliability of gefitinib as a single agent was not efficient in advanced HCC.[31,32] Moreover, targeting EGF pathway with lapatinib, as inhibitor of EGFR and HER2/NEU, showed no objective response other than disease stabilisation in a few patients.[26,33]

Agents Targeting IGF Signaling Pathway

Insulin growth factor (IGF) or insulin growth factor-1 receptors (IGF-1R)-targeted agents have been investigated for their therapeutic reliability in the treatment of HCC. Agents targeting insulin-like

growth factor (IGFR) pathway include monoclonal antibodies (e.g. OSI-906, BMS-554417) against IGF-1R and small molecule inhibitors (e.g. IMC-A12) are currently under evaluation in clinical trials of HCC patients.[34,35] The significance of the insulin-like growth factor pathway, as a therapeutic target for HCC treatment, is based on its essential role in the consequential activation of pathways common to EGFR, PI3K/Akt/mTOR-axis and Ras/MEK/ERK pathways.[36,37] The over-expression of IGF-II, IGF-1R and IRS are mostly associated with cell proliferation, inhibition of apoptosis as well as in tumour invasion.[38-40]

Agent Targeting c-Met Signaling Pathway

c-Met signaling pathway is an essential pathway for the development and progression of HCC, and is thereby considered a potential therapeutic target for HCC treatment. Previous and current clinical investigation of agents targeting this pathway showed a promising progression in the treatment of HCC patients.[41] Among these agents, foretinib (GSK136089) is one of the first multi-target c-MET TKI to undergo clinical investigation and produced objective response rate in HCC patients.[42] Also, other c-Met inhibitors like tivantinib (ARQ197) have been evaluated in sorafenib-failed HCC patients. More importantly, the clinical benefit of tivantinib in HCC patients is promising when compared with the other therapeutic agents.[41]

Agents Targeting Ras/Raf/MEK/ERK Signaling Pathways

The mitogen activated protein kinase (MAPK), Ras/Raf/MEK/ERK pathway is a potential therapeutic target for several agents with antitumour activity.[43,44] Dysregulation of the Ras/Raf/MEK/ERK pathway is associated with the development and progression of HCC. The continuous activation of Ras pathway has been demonstrated in HCC patients' samples as an evidence for the essential role of Ras in the process of hepatocarcinogenesis.[45,46] The activation of Ras/Raf/MEK/ERK pathway can be mediated in response to the up regulation of signaling pathways, such as IGF,[47] EGF,[48] VEGFR[49] and PDGFR.[50] Therefore, the inhibition of the Ras/Raf/MEK/ERK pathway using small molecules such as, sorafenib, selumetinib (AZD6244) and regorafenib is considered a relevant therapeutic strategy for HCC treatment. The multikinase inhibitor, sorafenib, has been shown to inhibit VEGFR-2,/-3, PDGFR-β, Flt-3 and c-Kit and subsequently downstream signaling pathways.[50-53] Sorafenib has been approved for the treatment of

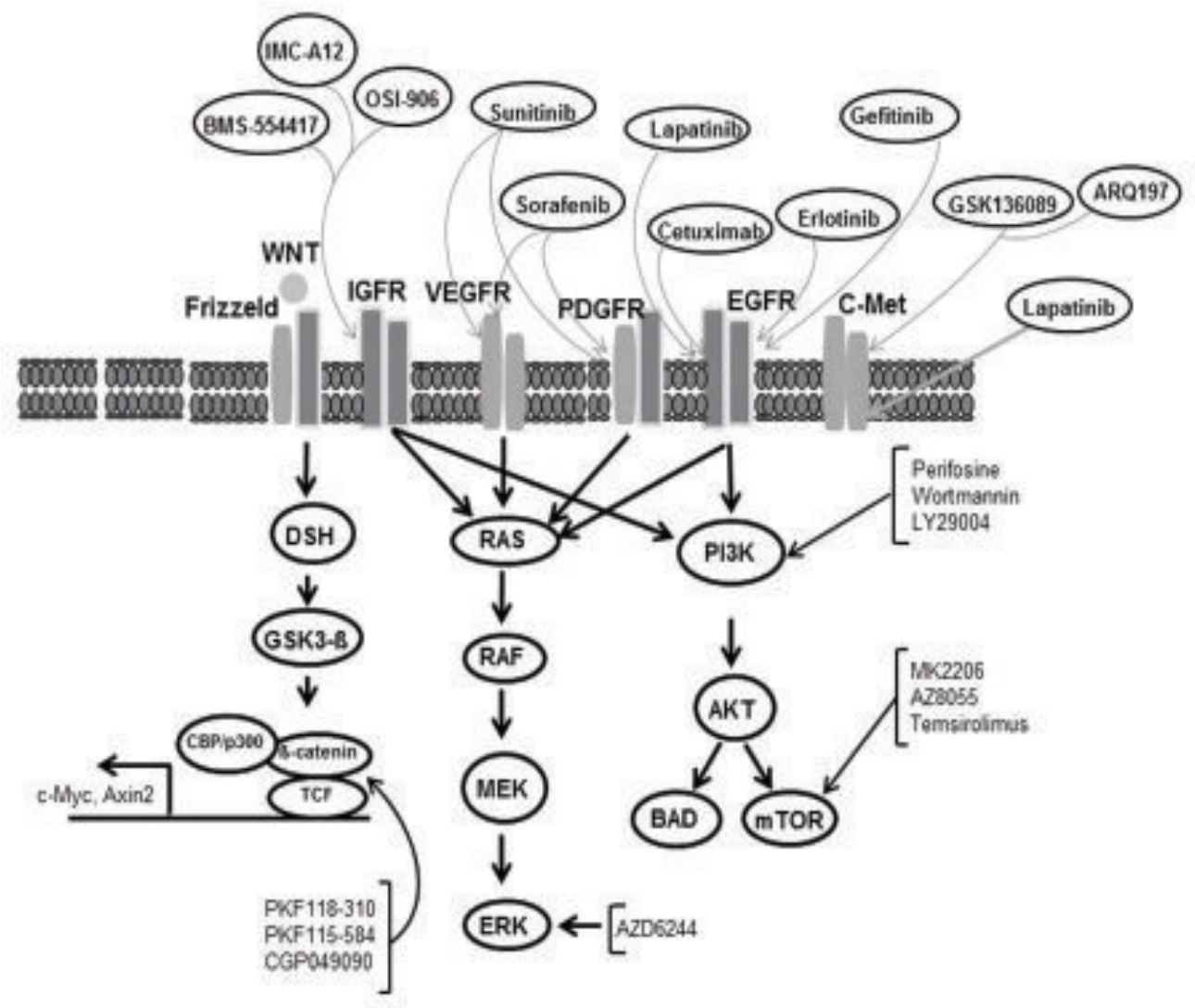

Figure 1. Signaling pathways and corresponding targeted therapies in HCC.

patients with advanced HCC.[54] Selumetinib (AZD6244), a further non-ATP-competitive small-molecule inhibitor of the mitogen-activated protein kinase MEK1/2, whose evaluation in patients with advanced or metastatic HCC confirmed its anti-HCC activity.[55,56] Also, multikinase inhibitor, regorafenib, which can target Raf, FGFR, c-Kit, VEGFR and PDGFR, has been evaluated for its safety and tolerability in patients with HCC who failed sorafenib treatment.[57,58]

Agents Targeting PI3K/Akt/mTOR Signaling Pathways

PI3K/Akt/mTOR is crucial in the development and progression of HCC and its activation results in a response to the ligation of growth factors to the membrane receptors including, EGFR and IGF-R1.[59,60] Thus, once activated, Akt becomes able to phosphorylate intracellular substrates, whose phosphorylation can lead to the promotion of cell survival in addition to the positive modulation of mTOR function.[61] Accordingly, some of PI3K inhibitors, such as perifosine, wortmannin and LY29004, have been analysed for their anti-HCC activity.[62,63] Despite their anti-HCC potential as shown in several preclinical studies, the clinical relevance of PI3K inhibitors has never been considered in any studies. Instead, other clinically relevant agents targeting key components of the PI3K/Akt/mTOR pathway have been intensively studied for their antitumour activity. These agents include MK2206, AZD8055 and temsirolimus (Kelley *et al*., 2013; Simioni *et al*., 2013; *et al*., 2013). MK2206 is an inhibitor of Akt that has the potential to alter the resistance of HCC patients to sorafenib.[64] Whereas, AZD8055 is an inhibitor of mTOR kinase that is investigated for its anti-HCC activity.[65,66]

Wnt-β-Catenin Pathway

The role of Wnt-β-Catenin pathway in the regulation of the biochemical processes of cell growth and differentiation is widely documented.[67,68] Genetic and epigenetic alteration of these pathways is mostly associated with development and progression of tumours, including HCC.[69,70] Thus, targeting the key components of this signaling pathway is considered a relevant therapeutic strategy for the treatment of HCC. Accordingly, several pharmacologic agents have been developed to target Wnt-β-catenin. These pharmacologic agents include PKF118-310, PKF115-584 and CGP049090.[71] The evaluation of the anti-tumour activity of these small molecules demonstrated their ability to antagonizthe TCF/β-catenin factor complex in vitro and in vivo model of HCC.[72] These small molecules mediate their anti-tumor activity by the induction of apoptosis together with the reduction of cyclin D1 expression.[71] Also, the reduction of the downregulation of the proto-oncoproteins such as, c-Myc and surviving in response to the treatment with these small molecules has been observed.

Conclusion

The potential of the signaling pathways as therapeutic target for tumour treatment has gained more attention in recent years. Hyperactivation of pathways associated with cell death or destruction of signaling pathways associated with cell growth and proliferation are relevant therapeutic strategy for HCC treatment. More importantly, the extensive research studies on key components of the aberrant pathways that are implicated in HCC development and progression may help to improve the current available therapies, and pave the way for the discovery of novel therapeutic targets. Understanding the mechanistic action of current therapies will lead to revolutionary changes and progress in the treatment management of HCC. Although several promising novel anti-HCC agents are under investigation and many molecularly targeted agents undergo different stages of clinical development and evaluation, a careful strategy for combining these clinically relevant agents is essential. The combination of different anti-HCC agents should be a key approach for improving the effectiveness and utilisation of anti-HCC agents. Future research will continue to uncover the mechanisms of the development and progression of HCC, and identify key molecular targets for HCC treatment. The field of the molecular target therapy in cancer has been established and HCC as tumour model will help encourage other researchers. Thus, future research in the field of HCC will increase the clinical benefit, reduce the expected adverse effects and minimise the cost of utilisation novel targeted anticancer agents.

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Sorafenib Therapy for Hepatocellular Carcinoma: From Evidences to Clinical Practice

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Introduction

Hepatocellular carcinoma (HCC) has an increasing incidence worldwide, and is the main cause of death in patients with cirrhosis. Despite application of screening and surveillance protocols for early detection HCC, and the recent therapeutic progress, prognosis and life expectancy is still poor, even when treatments have been considered as potentially radical.[1,2]

Natural history of HCC is severe and extremely variable, and prognostic factors influencing outcomes are incompletely defined. Data from literature showed that the 1- and 2-year survival rates ranged from 0-75% and from 0-50%, respectively (Figure 1).[3-5] These substantial differences in overall survival are due the presence of prognostic factor still unidentified and not included in current staging systems, resulting in their defective prognostic power.[6]

In the absence of an ideal prognostic model, treatment algorithms for patients with HCC in Europe and North America have been assessed on the basis of the Barcelona Clinic Liver Cancer (BCLC) classification.[7,8] Curative treatments for early-stage tumours include liver transplantation, resection and percutaneous ablation (radiofrequency ablation (RFA) or percutaneous ethanol injection (PEI)). Transarterial chemoembolisation (TACE) is indicated in asymptomatic patients with well-preserved liver function, and multinodular or large unresectable tumour, without macrovascular invasion or extrahepatic spread (ES) or at an early stage in patients for whom RFA or PEI cannot be performed because of tumour position (proximity to gallbladder, biliary tree or blood vessel), or due to the failure of previous curative treatments.

Patients with mild related symptoms and/or macrovascular invasion or ES fall in advanced stage. Previously, no effective therapy existed for the treatment of patients at this stage, a scenario that was subverted by the advent of sorafenib in 2007.[9]

Finally, subjects with cancer symptoms related to advanced liver disease (Child-Pugh C without transplant options), tumour growth with vascular involvement, ES or physical impairment (PS >2), are classified as end stage disease. This stage has a poor prognosis, with a median survival of 3-4 months, and best available supportive care (e.g. management of pain, nutrition and psychological support) is the only available therapy in this setting.

To note that as reported in many studies, complete radiological response after loco-regional treatments, as well as for surgical or systemic therapies, is a surrogate end-point which is strongly linked to overall survival. Therefore, imaging follow-up seems to play a crucial role in evaluating the effectiveness of HCC treatment.[10-12]

The aim of this article is to describe the role of sorafenib in the treatment of HCC and the current evidence regarding its efficacy, tolerability and safety in clinical practice.

Clinical Efficacy

Sorafenib (Nexavar®, Bayer Healthcare and Onyx Pharmaceuticals) is a multi-targeted orally active small molecule tyrosine kinase inhibitor (TKI) of Raf kinase, Platelet Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor (VEGF) and cKit.13 The originality of Sorafenib lays in its simultaneous targeting of the Raf/Mek/Erk pathway.

Already approved as a therapy for advanced renal cell carcinoma, it has been the first systemic agent to demonstrate a statistically significant

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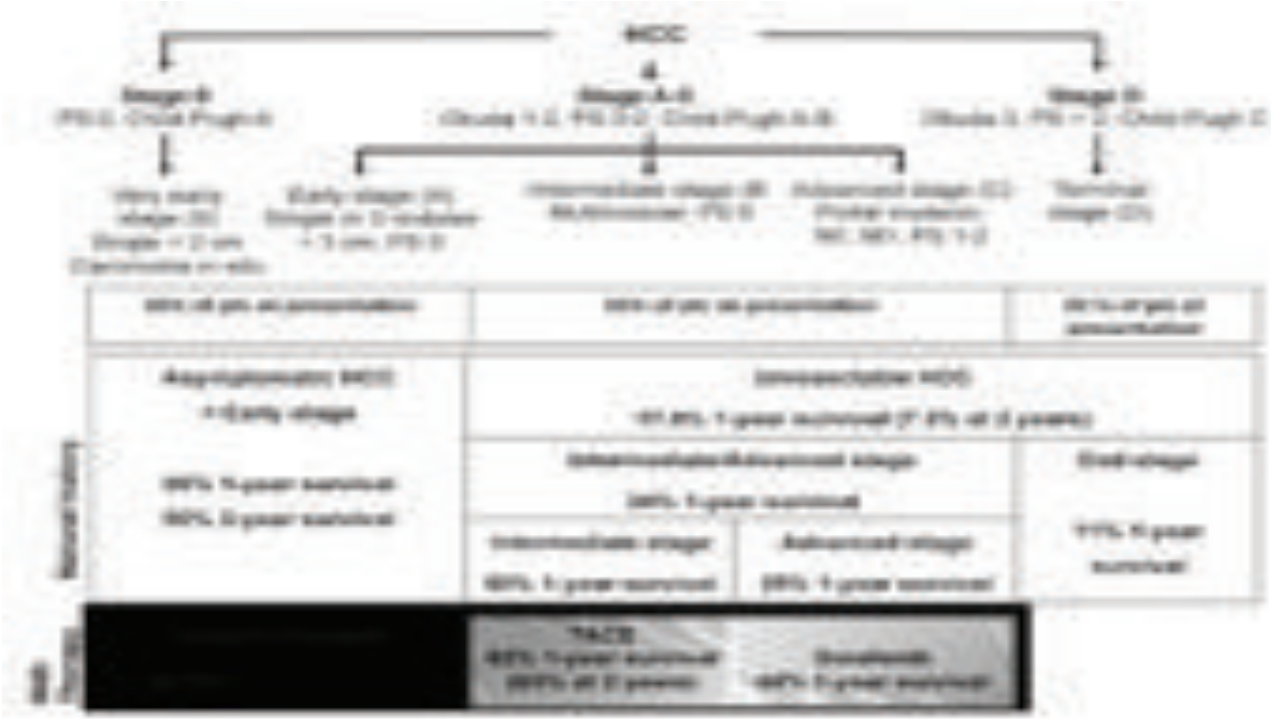

Figure 1. Natural history and treatment effects for hepatocellular carcinoma.[5]

improvement in the overall survival (OS) for patients with advanced HCC and well-preserved liver function Child-Pugh (CP) A, receiving a "Fast Track" designation by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) for the treatment of patients with unresectable HCC in 2007.

The drug has been studied in two large, multicentre, randomised controlled trials in patients with advanced, unresectable HCC.[9,14]

In the SHARP (Sorafenib HCC Assessment Randomized) study,[9] 602 primarily European patients were randomised to Sorafenib (400mg twice daily) or placebo. Patients with advanced disease were defined as being ineligible for, or having disease progression after, surgical or locoregional therapies. This trial was performed in predominantly Child-Pugh A and hepatitis C virus related liver disease, and provided a survival advantage for sorafenib (n=299) versus placebo (n=303) proving a 31% decrease in the risk of death, with a median survival of 10.7 months and 7.9 months, respectively (p<0.001, HR 0.69, CI 95% 0.55–0.87). Sorafenib was also associated with a significant improvement in time to progression (TTP) with a median of 5.5 months vs 2.8 months for placebo arm (p<0.001, HR 0.58, CI 95% 0.45–0.74). Discontinuation of treatment because of adverse events, dose reduction and permanent treatment discontinuation occurred in 38%, 26% and 11% respectively, showing a modest safety profile.[9]

The efficacy of Sorafenib in Asian population was assessed in a second placebo-controlled phase III trial performed on 226 patients with Child-Pugh A cirrhosis and no prior systemic therapy for HCC receiving Sorafenib 400mg twice daily or placebo.[14] The study showed a significantly better overall survival with a median of 6.5 versus 4.2 months (p=0.014, HR 0.68, CI 95% 0.50–0.93) and a better TTP of 2.8 versus 1.4 months (p=0.0005, HR 0.57, CI 95% 0.42–0.79) both for Sorafenib arm.

Despite this study showing a similar safety profile with respect to the SHARP trial, the amount of benefit was found to be globally inferior. In fact, the treated group in the Asian trial showed a shorter survival respect to the one of control group in the SHARP (6.5 vs 7.9 months), although both trials used the same entry criteria. Nevertheless, patients enrolled in the Asian study were sicker at the beginning compared with those of the SHARP trial, with a worse performance status (PS) and more advanced stage of disease.[14]

Sorafenib in Real Clinical Practice: Efficacy, Safety and Tolerability

Although RCTs provide the highest level of evidence for the rigorous design and the precise evaluation of the efficacy in a well-defined population, they often include a population of patients that do not closely reflect the clinical practice, and they are generally underpowered to evaluate drug tolerability and safety due to rigid protocols and strict exclusion criteria. On the other side, despite the presence of several biases, observational studies allow to properly unravel the clinical benefit and toxicity of a drug, like Sorafenib, when administered in real life patients carrying co-morbidities.

This is particularly relevant considering that, since the registration trial[9] was prematurely stopped at the second interim analysis due to

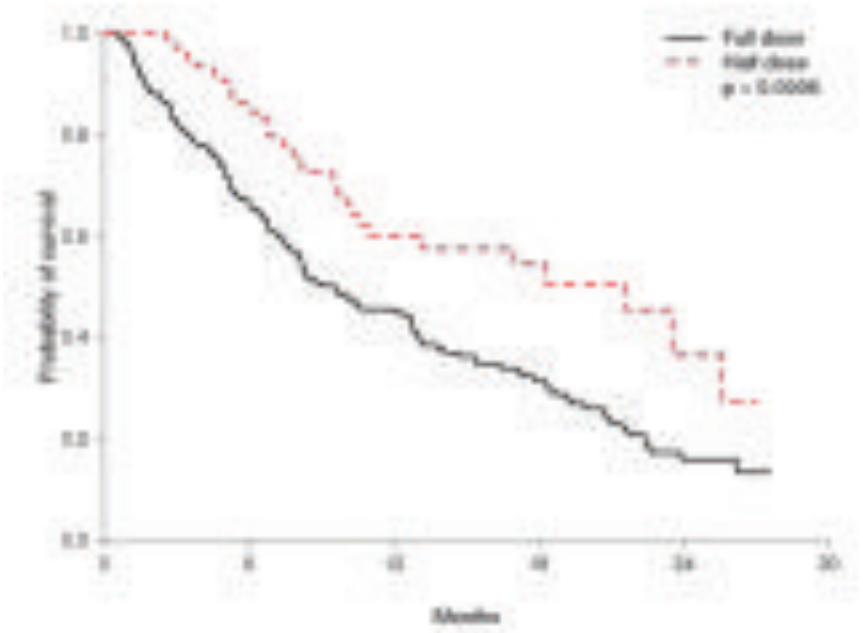

Figure 2. Kaplan–Meier analysis of overall survival in 296 hepatocellular carcinoma patients according to the prevalent dose of Sorafenib (219 full dosed or half dosed <70% treatment period and 77 half dosed >70% treatment period) in the SOFIA study.[17]

significant survival advantage in the active drug arm, benefits and risks of Sorafenib therapy could not be adequately assessed.

In this regard, it is well known that RCTs stopped early for benefit can overestimate the magnitude of the treatment effect and underestimate the drug risks.[15,16]

With this in mind, the Sorafenib Italian Assessment (SOFIA) study, a multicentre, prospective, observational, non-interventional study, has been performed with the aim to assess the safety and effectiveness of Sorafenib in real clinical practice.[17,18]

In all, 296 patients with advanced HCC or intermediate HCC who were not eligible to/or failure of ablative therapies, were consecutively evaluated. A total of 260 (88%) patients were in the Child-Pugh A class. None had ascites, clinically overt jaundice or hepatic encephalopathy. At baseline, 115 patients (39%) had macroscopic vascular invasion by the tumour, whereas 104 (35%) had extrahepatic spread of the tumour. Overall, 222 (75%) patients were in BCLC-C stage and 74 (25%) in BCLC-B stage, including 26 (35%) who were unfit for locoablative treatment. Sorafenib treatment was interrupted in 103 (44%) for disease progression, in 95 (40%) for an adverse event and in 38 (16%) for liver decompensation. By Kaplan-Meier test, the median survival was 10.5 months in the overall cohort, 8.4 months in BCLC-C versus 20.6 months in BCLC-B patients ($p<0.0001$), and 21.6 months in the 77 patients treated for 70% of the time with a half dose versus 9.6 months in the 219 patients treated for <70% of the time with a full dose (Figure 2). ECOG PS, macrovascular invasion, extrahepatic spread of the tumour, radiologic response at month 2, and full dose sorafenib were independent predictors of shortened survival.

Overall, the SOFIA study[17] confirms the effectiveness of Sorafenib with a lower safety profile than that of the SHARP trial,[9] also showing that a significant proportion of patients needed dose adjustment of Sorafenib. In particular, the overall incidence of AEs was 91%. Fatigue, weight loss, diarrhoea and hand-foot skin reaction (HFSR), were the most frequent AEs. Moreover, it was reported 18% occurrence rate of hypertension (both *de novo* or as a worsening of a pre-existing condition) that frequently led to dose reduction or to discontinuation of therapy, compared with 5% observed in the first phase III study. Overall, discontinuation of Sorafenib because of adverse events occurred in 40% of patients. Moreover, the cost-effectiveness analysis of Sorafenib treatment in field practice based from the SOFIA study, showed that in daily practice dose-adjusted, but not full-dose, is a cost-effective treatment compared to BSC in intermediate and advanced HCC.[19]

Currently, the safety of sorafenib in specific subgroups, especially in Child-Pugh B patients that were not well represented in RCTs, is still being evaluated in the Global Investigation of therapeutic DEcisions in hepatocellular carcinoma and Of its treatment with sorafenib (GIDEON), a global, non-interventional surveillance study, evaluating the safety of Sorafenib in all patients with unresectable hepatocellular carcinoma under real-life practice conditions.[20]

Finally, despite the skepticism regarding the administration of systemic chemotherapy in the elderly, recent studies demonstrate that treatment with Sorafenib is effective regardless of age, with a potential reduced tolerance in older patients, which require more frequent dose adjustments and a closer follow-up.[21]

Combination Therapies Including Sorafenib

As mentioned earlier, Sorafenib is the current reference standard for the treatment of advanced HCC patients.[9,14]

Due to its original mechanism of action, able to reduce the blood supply to the tumour, a growing interest in assessing the potential of Sorafenib in the adjuvant setting exists, eg after curative treatments after which the potential post-treatment residual tumour may increase in volume and resume its growth after having recovered its blood supply, making it plausible the usefulness of an anti-angiogenic drug.

The Sorafenib as Adjuvant Treatment in the Prevention of Recurrence

of Hepatocellular Carcinoma (STORM) trial, a randomised, double-blind, placebo-controlled, Phase III multicentre study, has been performed with the aim to evaluate the effectiveness of Sorafenib versus placebo as adjuvant therapy in patients with HCC following surgical resection or local ablation. The primary endpoint of the study was recurrence-free survival, while secondary endpoints included time to recurrence and overall survival. More than a thousand patients were randomised to receive either 400mg of Sorafenib twice daily or matching placebo for four years or until disease recurrence. Unfortunately the recent final analysis showed that the trial did not meet its primary endpoint.[22]

Sorafenib has also been investigated in the setting of combination non-curative therapies. Combination of TACE and Sorafenib has been extensively explored in the last years, based on experimental evidence that suggests how local hypoxia resulting by TACE produces a temporary increase in levels of VEGF. In this regard, the SPACE trial, a large, randomised, double-blind, placebo-controlled phase II study, showed that regimen with DEB-TACE and administration of Sorafenib has a good safety profile with a better performance, in terms of TTP and time to vascular invasion or extrahepatic spread, when compared with DEB-TACE alone.[23] However, this promising result needs to be confirmed in a phase III setting.

Finally, Sorafenib is currently tested in RCTs in combination regimens with other anticancer drugs, as first-line therapy in the treatment of advanced HCC, for example Sorafenib with or without doxorubicin hydrochloride, and Sorafenib with or without tegafur-uracil.[22]

Unfortunately, despite intensive investigation, to date, no evidence supports the use of Sorafenib in combination with other anticancer therapies.

Conclusions

The identification of survival benefits of sorafenib against advanced HCC opened a new challenge to improve knowledge regarding molecular features of HCC carcinogenesis, in order to develop new molecular targets and to determine optimal therapeutic combinations. Several signaling pathways have been identified and the role of some angiogenic factors (EGF, VEGF and PDGF) in HCC carcinogenesis has been established. In the near future subclassify HCC patients according to their genomic and proteomic profiling will be necessary.[24] In fact, defining combined targeted agents effective for a specific subclass will hopefully lead to personalised medicine. Waiting for data on pharmacokinetic/pharmacodynamic analysis in individual patients, adjusted-dose Sorafenib may have implications for personalised therapy, especially in "fragile" patients such as elderly ones and those with comorbidities. Interestingly, a recent study showed a positive correlation between survival and dermatologic adverse events, suggesting that as already reported for other tyrosine-kinase inhibitors, adverse events occurring during Sorafenib treatment for HCC may be an efficient clinical marker for predicting survival.[25]

Given the complexity of the disease, in particular for the presence of chronic liver disease and the large number of potentially useful therapies, a multidisciplinary management of the disease is strongly recommended and personalised treatment decisions should be discussed among tumour board meetings, inside which the position of the hepatologist assumes a central role due to the relevance of liver status at baseline and during therapy.

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The Role of the Liver in Insulin Resistance

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During the EASL meeting 2014 in London, Yamazaki and colleagues reported a significant increase in diabetes occurrence in patients with baseline non-alcoholic fatty liver disease (NAFLD) compared to patients without liver steatosis.[1] This first oral communication of the 'fatty liver session' opened the interesting debate on the interactions between the liver and insulin sensitivity. The aim of this review is to discuss the role of the liver in insulin resistance, locally (hepatic insulin resistance) and in distant organs, the link between liver steatosis and hepatic insulin resistance and the possible other complications of NAFLD.

Central Role of the Liver in Insulin Resistance Pathogenesis

Hepatic Insulin Resistance: What Does it Mean?

The liver is an insulin-sensitive organ that produces more than 80% of the endogenous glucose,[2] the rest of gluconeogenesis taking place in the kidney.[3] The role of insulin is to regulate the whole body glucose homeostasis. Insulin resistance refers to a condition in which regular amounts of insulin are not able to induce their effect in insulin sensitive tissues (the muscles, the adipose tissues, the brain, the kidney and the liver). It includes inability of insulin to induce glucose uptake in peripheral tissues (adipose tissue and muscles), inability of insulin to decrease adipose tissue lipolysis, inability of insulin to decrease appetite via its action on the hypothalamus, inability of insulin to suppress the kidney glucose production and inability of insulin to block production of glucose and very low density lipoproteins by the liver. Maintenance of hepatic glucose production in the face of hyperinsulinaemia is called hepatic insulin resistance.[4,5] It represents the major determinant of fasting hyperglycaemia.[2]

Which Methods to Evaluate Hepatic Insulin Sensitivity?

We will focus on insulin sensitivity in terms of glucose metabolism (and not fatty acids metabolism). As mentioned above, the liver, the muscle and the adipose tissue are the three main insulin-sensitive organs (Figure 1) and usual methods to assess insulin sensitivity explore the full body sensitivity without making any distinction between those tissues. Elevated fasting plasma glucose and/or fasting plasma insulin levels indicate insulin resistance. Indices calculated from these parameters (such as homeostasis model assessment of insulin resistance (HOMA-IR) or quantitative insulin sensitivity check index (QUICKI) provide good indirect assessment of global insulin resistance. Oral glucose tolerance tests evaluate the dynamic response to a glucose load and integrates reactive insulin secretion and insulin sensitivity. The only tests that directly measure global insulin sensitivity are the insulin sensitivity test and the hyperinsulinaemic-euglycaemic clamps (without tracer). In the insulin sensitivity test, the glucose lowering effect of a given dose of insulin is directly evaluated. With the clamp technique, insulin is infused constantly and the rate of the parallel glucose infusion necessary to maintain normal blood glucose determines the level of insulin sensitivity. The assessment of the specific liver insulin sensitivity can be obtained *in vivo* by the hyperinsulinaemic-euglycaemic clamp combined with the perfusion of marked glucose, to make the distinction between the endogenous (liver) non-labelled glucose production and the exogenous (labelled) glucose administration. Using the glucose infusion rate and the hepatic glucose production, the rate of glucose disappearance (glucose turnover) reflecting peripheral insulin sensitivity can be calculated.[2] The direct evaluation of the hepatic glucose production by the arteriovenous-difference technique (from the difference between arterial and venous glucose concentration in liver blood flow) is also described.[3] However, it is not used in clinical practice, requiring complicated catheterisation of the liver. Furthermore, it is not reliable, the liver receiving blood flow from the portal vein and the hepatic artery.[2]

Nicolas Lanthier received his medical degree in 2005 from the Université catholique de Louvain, Belgium. He received his Masters degree in Gastroenterology in 2011 and his PhD degree in 2013 for his thesis entitled Hepatic insulin resistance: role of macrophages/Kupffer cells. He is currently both clinical hepato-gastroenterologist at the Cliniques Universitaires Saint-Luc in Brussels and researcher at the Laboratory of Hepato-gastroenterology of the Université catholique de Louvain. His work is focused on hepatic metabolic diseases, alcoholic liver disease and liver regeneration. He is a member of the Société Royale Belge de Gastro-entérologie (SRBGE) and the European Association for the Study of the Liver (EASL).

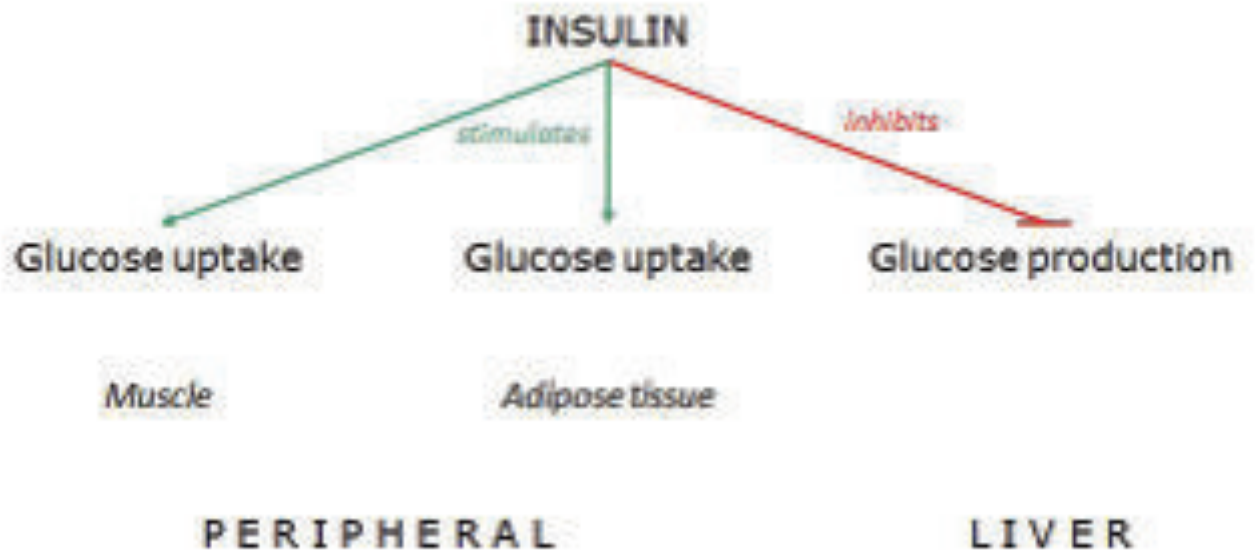

Figure 1. The effect of insulin on glucose metabolism of the three main insulin sensitive tissues.

On a liver biopsy, indirect indices of liver insulin sensitivity can be obtained and used to compare two groups of patients. The levels of proteins and phosphoproteins of the signaling cascade in response to insulin (insulin receptor, insulin receptor substrate,…)[6] and the hepatic measurement of gluconeogenic enzyme (phosphoenolpyruvate carboxykinase and glucose-6-phosphatase) mRNA expression or protein level[7] are also possible. Advantages and limitations of all techniques are summarised in Table 1.

Hepatic Insulin Resistance: The First Step of Diabetes?

In a pro-inflammatory and increased hepatic lipid content context, there is an activation of stress kinases converging to alter insulin signaling pathways (decreased amount of liver insulin receptors as well as decreased activation of down-stream insulin receptor signals) and enhance hepatic glucose production.[6,8,9]

Collectively, experimental time-points studies in rodents revealed that under a high-fat diet, insulin resistance appears first in the liver after a few days, then in peripheral tissues after a few weeks.[10-14] Interestingly, this rapid hepatic insulin resistance development occurs before the appearance of overt hyperinsulinaemia.[12,15] In humans, the same rapid hepatic insulin resistance is evidenced under high fat diet. Indeed, a short-term (5 days) fat overfeeding increased hepatic glucose production and fasting glucose level without affecting peripheral insulin action.[16] In addition, deletion of the insulin receptor specifically in hepatocytes but not in skeletal muscles leads to hyperglycaemia.[17,18] Thus, hepatic insulin resistance causes ill-adapted glucose production responsible for hyperglycaemia and constitutes thereby the first step of diabetes.

Role of the Liver in Peripheral Insulin Resistance Occurrence: The Emerging Impact of Hepatokines

The liver is thus the first target of metabolic disruption and it may participate to metabolic and inflammatory peripheral organ alteration that characterises the metabolic syndrome. Data on hepatitis C patients support this view. While the virus infects only hepatocytes (and not muscle cells), hepatitis C patients are characterised by liver and muscle insulin resistance.[19] Also, viral clearance leads to reduced incidence of type 2 diabetes development.[20] The impact of the liver and hepatic related proteins on peripheral insulin sensitivity has emerged and is supported by several lines of experimental evidence. Liver-specific activation of inflammation in mice induces both hepatic and systemic insulin resistance.[21] In humans, data evidenced an increased liver derived secretory protein called selenoprotein P in diabetic patients.[22] Another liver protein called fetuin A was also demonstrated to be increased in diabetes[23] but also in prediabetic states in a large prospective study.[24] In experimental conditions, those liver proteins, called hepatokines (in parallel to adipokines from the adipose tissue), are able to induce insulin resistance in peripheral tissues such as the adipose tissue[25] or the muscle.[22]

Collectively, those data are consistent with the fact that the liver and liver derived products beside their role on hepatic gluconeogenesis, are also able to affect peripheral insulin sensitivity.

Steatosis: The Link Between The Liver and Insulin Resistance and its Importance in Clinical Practice

Liver insulin resistance is difficult to dissociate from fatty liver, i.e. fat accumulation within lipid droplets exceeding 5% of the liver mass.[26]

Yet, whether insulin resistance causes or is a consequence of fatty liver is still debated.

Fatty Liver, Metabolic Syndrome and Insulin Resistance

Usually, hepatic steatosis is described as the hepatic manifestation of the metabolic syndrome, in which insulin resistance is the corner stone and defined by the presence of abdominal obesity associated with 2 of the following elements: elevated blood pressure, elevated triglycerides, low high-density lipoprotein cholesterol or hyperglycaemia (Table 2).[27] The quantification of liver fat by proton magnetic resonance in non-diabetic subjects indicates that liver fat is 4-fold higher in subjects with metabolic syndrome compared to subjects without.[28] While overnutrition, soft drink consumption and reduced physical activity are the main causes of hepatic fat accumulation, called non-alcoholic fatty liver disease,[29-31] other factors such as alcohol,[32] hepatitis C[33] and lipodystrophy[34] may also cause steatosis and insulin resistance.

Adipose tissue insulin resistance causes increased delivery of fatty acid to the liver (due to the uncontrolled adipose lipolysis) that participates to steatosis.[35] However, in population studies, the presence of hepatic fat is suggested to constitute one parameter developing before the apparition of type 2 diabetes[36] and, more than visceral fat, is associated with insulin resistance in non-diabetic subjects,[28,37] individuals at risk for diabetes,[38] obese people[39] and diabetic patients.[40] A moderate (8%) weight loss in diabetic patients improves glycaemic control, attributable to hepatic gluconeogenesis normalisation (proved by clamp studies) associated to a dramatic reduction in hepatic lipid content.[41] Those findings suggest that, rather than being its consequence, steatosis may cause hepatic insulin resistance and that the liver plays a central role in glucose homeostasis.

Method	Advantage(s)	Limitation(s)
Direct measures of insulin sensitivity		
Clamp	Gold standard In vivo measurement of HGP and glucose utilisation by peripheral tissues if tracer used	Time-consuming Experienced centre needed Aggressive (catheter, radiolabelled glucose)
Insulin suppression test	First method to assess insulin sensitivity in vivo	No data about specific HGP Risk of hypoglycaemia Time-consuming
Indirect measures of insulin sensitivity		
Glucose or insulin levels	Inexpensive Easy to do	Depending also on insulin secretion and on beta cell function No data about HGP
Oral glucose tolerance test	Simple for large trials	No data about HGP Time-consuming
HOMA-IR Insulin x glucose /22.5	Good correlation with the clamp Simple Decrease insulin fluctuations by 3 blood samples	No data about specific HGP
QUICKI 1/ [log(insulin) + log(glucose)]	Good correlation with the clamp Simple	No data about specific HGP
Hepatic insulin signaling cascade analysis	Specific for the liver Evaluation of the level of insulin resistance (receptor or downstream)	Liver tissue needed Comparison group needed
Gluconeogenic liver enzyme	Specific for the liver	Liver tissue needed Comparison group needed Correlation with hepatic insulin sensitivity under debate
Liver glucose arteriovenous-difference technique	Reflect of the hepatic glucose production	Invasive Poorly reliable

Table 1. Current approaches assessing insulin sensitivity. HGP, hepatic glucose production.

Increased waist circumference, with ethnic-specific cut-offs (for white people of European origin: men ≥94 cm, women ≥80 cm)
And any two of the following:
Triglycerides >150mg/dL or treatment for elevated triglycerides
HDL cholesterol <40mg/dL in men or <50mg/dL in women, or treatment for low HDL
Systolic blood pressure >130mmHg, diastolic blood pressure >85mmHg, or treatment for hypertension
Fasting plasma glucose >100mg/dL (5.6mmol/L) or diagnosis of type 2 diabetes

Table 2. Metabolic syndrome criteria (International Diabetes Foundation).

Does Fatty Liver Mean Insulin Resistance?

The presence of liver fat is associated with a degree of hepatic insulin resistance in humans even in lean subjects.[37] However, the level of systemic insulin resistance also depends on peripheral insulin sensitivity, explaining a slight variability in the correlation between liver fat content and systemic insulin resistance.[29] Furthermore, it has to be noted that dissociation between liver fat and insulin resistance is demonstrated in rodents and humans with genetic disorders leading to triglyceride accumulation in the liver. Transgenic mice with overexpression of one triglyceride synthesis enzyme[42] or in the absence of long-chain fatty acids elongation[43] develop steatosis without insulin resistance. The enhancement of fatty acid oxidation in obese mice corrected insulin resistance without affecting steatosis.[44] In humans, patients with familial abetalipoproteinaemia also present high hepatic triglyceride content without hepatic insulin resistance.[45] Patients with genetic PNPLA3-mediated liver triglyceride accumulation have also a metabolically 'benign' fatty liver, not associated with insulin resistance.[46]

Current hypothesis regarding those observations is that fatty acids excess in the liver (in NAFLD due to overnutrition) leads to a local inflammatory response, called lipid-mediated toxicity or lipotoxicity, associated with hepatic insulin resistance, and that triglycerides are even less toxic than fatty acids or may protect from their lipotoxic effects.[29]

In the same way, experimental data in mice evidenced a dissociation between fatty liver and insulin resistance if local inflammatory macrophages (Kupffer cells)[12] or inflammatory pathways[21] are suppressed, suggesting that hepatic fat itself, without concurrent inflammation, is not always deleterious for the liver.

Liver Related Complications of NAFLD

Thirty percent of adults in the United States have steatosis.[47] Long term considered as a benign condition, this fatty liver is now recognised as a major health problem, due to liver and to general complications. First, as mentioned above, the steatotic liver is associated with hepatic insulin resistance, which is a cause of hyperglycaemia.[48]

Second, steatosis can also evolve in 10% of patients in a more aggressive, inflammatory and fibrotic disease, called non-alcoholic steatohepatitis (NASH) which has a risk of cirrhosis.[47] Even in the absence of cirrhosis, hepatocellular carcinoma (HCC) may complicate steatosis with mild or absent fibrosis.[49] The term 'Non-alcoholic fatty liver disease (NAFLD)' includes the wide spectrum of liver damage, ranging from simple steatosis to steatohepatitis and advanced fibrosis. The progression between simple steatosis to NASH is correlated with the importance of insulin resistance.[6]

Third, steatosis increased the risk of fibrosis development of other diseases, such as hepatitis C virus or hepatitis B,[50,51] alcoholic liver disease[52] and haemochromatosis.[53] Insulin resistance itself has also been shown to directly predict fibrosis in hepatitis C patients.[54] Fourth, several clinical trials have demonstrated that liver failure related to primary graft non-function is related to donor fatty liver disease.[55,56]

General Related Complications of Fatty Liver

As mentioned above, systemic insulin resistance and diabetes mellitus can develop in the setting of NAFLD.[1,36] Furthermore, NAFLD patients have an increased overall mortality due to associated malignancies and ischaemic heart diseases,[57,58] the presence of hepatic steatosis being an independent supplemental risk factor for cardiovascular complications probably through the systemic release of inflammatory products playing a role in atherogenesis.[59-61] For malignancies, it is known that inflammatory cytokines such as TNF-α or IL-6 increased in insulin resistance are also potent activators of oncogenic pathways.[49] Hepatic steatosis is associated with colorectal adenomas,[62] colorectal cancers[63] and the severity of NAFLD correlates with precursor lesions of colorectal cancers.[64]

Conclusion

In conclusion, hepatic steatosis is usually presented as the hepatic manifestation of the metabolic syndrome. However, population studies and experimental data show that liver fat and liver insulin resistance (unrefrained liver glucose production) constitute early consequences of overfeeding and may predict the development of systemic insulin resistance, independently of body mass index and stronger than the presence of visceral fat. Liver steatosis has not to be interpreted only as a possible consequence but also as an active player of metabolic syndrome disturbances. In this context, the production of inflammatory proteins (called hepatokines) by the steatotic liver can be seen as a novel biomarker tool as well as future therapeutic targets for insulin resistance and other NAFLD associated complications.

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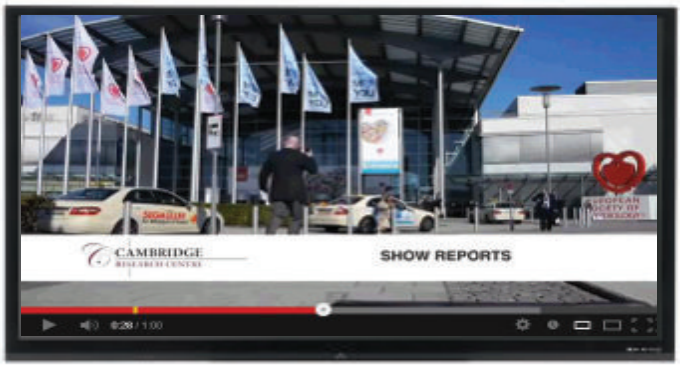

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Liver Metastasis from Non-colorectal Non-neuroendocrine Tumours

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Hepatic resection offers the only chance of long-term survival for patients with colorectal liver metastasis (CLM). The 5-year survival rate for these patients has increased from around 30% two decades ago to nearly 60% in 2014 and the 10-year overall survival rate is 20–25%. These advances are down to improvements in surgical technique and the use of new chemotherapeutic agents.[1] So the benefits of liver resection for CLM and also for neuroendocrine tumours are widely accepted.

After the colon, and in descending order of frequency, the pancreas, breast, ovary and stomach are the main sources of liver metastasis (LM). CLM have a less aggressive clinical and biological behaviour than metastases from other solid tumours like breast or lung; in the latter cases, the liver metastasis is considered another site of systemic disease. The role of liver resection for liver metastases from non-colorectal non-neuroendocrine (NCRNNE) tumours is still under discussion, but the positive results reported in a small number of recent studies support surgical resection in selected patients. In a comparative analysis, Reddy *et al.* reported no differences in CLM and NCRNNE LM, and found that long-term outcome after resection of NCRNNE was nearly equivalent to CLM.[2] A retrospective study of 1,452 patients by Adam et al demonstrated that liver resection for NCRNNE hepatic metastases is safe and effective.[3]

But the evidence in favour of liver resection of NCRNNE LM is insufficient. Studies of NCRNNE liver metastases often include neuroendocrine tumour patients in their analysis, which alters the survival data. In general, the series are highly heterogeneous; they include patients with NCRNNE hepatic metastases from several primary malignancies with different behaviours and sensitivity to chemotherapy, variable frequency of isolated hepatic metastases, and different lengths of disease-free interval between the resection of the primary tumour and the diagnosis of LM. So these variations in the presentations of the data prevent us from drawing reliable conclusions. Moreover, many studies include recruitment periods of 10 years or more, and during this time the chemotherapy drugs available may well change drastically.

There are no clearly defined prognostic factors for NCRNNE LM, but disease-free interval from the primary tumour and histological type are considered crucial factors. A period of 12 months was classically considered as the generic minimum period between primary tumour and LM to give an indication for surgery. A time interval longer than a year is a positive prognostic factor; in contrast, a longer disease-free interval is believed to indicate less aggressive tumour biology4. Adam et al classified NCRNNE LM outcome according to primary tumour sites and histologies as follows: favourable (adrenal, testicular, ovarian, small bowel, ampullary, breast, renal and uterine tumours); intermediate (gastric adenocarcinoma, exocrine pancreas, cutaneous melanoma, choroidal melanoma and duodenal tumours); and poor (gastro-oesophageal junction, pulmonary, oesophageal and head and neck tumours).[3]

Summarising by Type of Tumour

Liver Metastases from Breast Cancer (LMBC)

Patients with visceral metastases from breast cancer have a poor prognosis. LM are only very occasionally the only sites of systemic disease and extremely rare as solitary metastases. The liver is the sole site of metastasis in only 3% to 11% of breast cancer patients, but in the case of disseminated disease 65% of breast cancer patients present LM5. Without resection, long-term survival is the exception, varying

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from 1 to 15 months.[5-8] Few series of liver resection in LMBC have been published,[6-8] comprising around 500 patients operated at a few highly specialised centres.

Several questions regarding LMBC remain unresolved:

- Which chemotherapy regimens should be given before and after surgery? Response to chemotherapy appears to be an important predictor of survival following hepatic resection for LMBC. Patients who remained stable or progressed on prehepatectomy chemotherapy were 3.5 times more likely to die than responders. There is currently no consensus regarding the optimal protocol.

- What is the best timing for liver surgery? Liver surgery should be offered to all patients with a good performance status, stable disease with predictable resection with uninvolved margins, and with a long disease-free interval.

- Should hepatectomy be performed in patients with extrahepatic disease? Liver resection should be performed when bone metastases are the only extrahepatic disease but there is no consensus for other locations.

- Timing in patients with synchronous tumours? To consider surgery, liver disease must first be stabilised with chemotherapy.

Several prognostic factors have been identified in the series published.[9] The most frequently recorded are: prolonged tumour-free interval (1-2 years) between breast cancer surgery and liver metastasis diagnosis and absence of lymph node involvement at the time of breast cancer.

Liver resection in LMBC in highly selected patients may function as a cytoreductive rather than a curative procedure, because LMBC is considered as a disseminated disease. As a cytoreductive approach liver resection improves the survival rate, although disease-free time is usually brief.

Liver Metastases from Melanoma

Up to 30% of patients with melanoma develop distant metastases. After the lungs and brain, the liver is the third most frequently involved organ. Previously considered as a rare condition, autopsy studies have shown liver metastases in 55%-75% of the patients with melanoma.[10,11] Diagnosis is generally made by imaging studies during follow-up. PET/CT (well-known acronym) seems to be the best diagnostic method, with a sensitivity of 85%, a specificity of 100% and a positive predictive value of 98%.[13] Median survival in patients with LM without resection ranges between 2 and 6 months.[14] Palliative radiotherapy and systemic chemotherapy do not prolong survival and, although interferon-α and interleukin-2 have yielded promising response rates, no increase in survival has been reported.[15] Liver resection achieves 5-year survival rate between 5% and 25%. Surgery is cytoreductive, not curative.[16] Positive prognostic factors are a R0 resection, a solitary metastasis and absence of extrahepatic disease.[16]

Liver Metastases from Gastric Cancer

Indications for liver resection for LM from gastric cancer have not been established. Frequently, when LM appear, patients are already at an advanced stage of disease with a mean survival of less than six months. Some small series have shown an increased survival benefit with resection, reaching 5-year survival rates of 20%-42%. The following positive prognostic factors have been identified: solitary tumour or no more than three nodules, size less than 5cm, unilobular distribution, resection margin more than 10mm and disease-free interval between gastric cancer resection and liver metastases over one year.[17]

Liver Metastases from Lung Cancer

Liver resection for lung cancer metastases is rare. The few cases reported in the literature are usually included with other kinds of LM in large series. In patients with one or two liver nodules and more than one year of disease-free interval after the resection of primary tumour, hepatic resection may be an option.[18] Liver involvement is managed surgically only in these exceptional circumstances.

Gastrointestinal Stromal Tumour Liver Metastases

Gastrointestinal stromal tumours (GIST) are the most common mesenchymal neoplasms of the gastrointestinal tract and account for 1%-3% of all gastrointestinal malignancies.[19] The liver is known to be a common metastatic site for GIST, and previous studies have reported that 55%-72% of patients develop hepatic metastasis following complete resection of the primary tumour.[20]

Surgical resection with curative intent for hepatic metastases from GIST should be considered when feasible, as it may improve survival outcomes in selected patients. This seems especially true for patients with small single metastases and a long disease-free interval (>2 years).[21] A recent report concludes that combination therapy for GIST LM comprising surgical resection and tyrosine kinase inhibitors therapy is more effective than surgery or therapy alone.[22]

Liver Metastases from Ovarian Cancer

The liver is rarely the only site of metastatic ovarian cancer. Ovarian cancer can involve the liver through peritoneal lesions on the surface of the liver or with intraparenchymal metastases.[23,24] True LM, as opposed to peritoneal implants in the liver, usually appear in

patients with disseminated disease, and so liver resection is rarely performed. In some patients with limited extrahepatic disease or liver disease alone, liver resection is a safe alternative and improves the oncological results.[24]

Renal Cell Carcinoma and Urothelial Cancer

The data available on hepatic resection for renal cell cancer (RCC) metastases are limited to case reports. The liver is the sole organ involved in only 5% of metastatic RCC patients. In these patients, liver involvement is present in 20% of the patients and overall 1-year survival rate is below 10%. Alves *et al.* reported 46 liver resections for metastatic RCC patients with a 5-year survival of 13%.[25] With these limited data, we cannot routinely recommend hepatectomy in LM from RCC. In this situation, the use of sunitinib or sorafenib as neoadjuvant or post-hepatectomy therapy may improve outcome.

Conclusions

No randomised clinical trial about this topic has been published, but in selected patients, NCRNNE LM resection could improve survival rate comparing with only chemotherapy or other treatments. Histological type and disease-free interval from the primary tumour seems to be the most crucial prognostic factors. Patients that show disease progression receiving chemotherapy are bad candidates for resection. In some tumours such as breast cancer, resection of LM should be understood as cytoreductive surgery in patients with disseminated disease. Tailoring decisions in these patients is crucial.

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Current Strategies to Prevent Blood Loss During Liver Transplantation

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Advances in surgical techniques, perioperative care and improvements in immunosuppressive regimens have rendered liver transplantation (OLT) a successful therapy for patients with end-stage liver disease. However, perioperative blood loss is always a central issue and a considerable percentage of transplant recipients still have important transfusion requirements. Massive bleeding complications during OLT, though rare, still remains a major cause of morbidity and mortality.

Due to the well-known strict correlation between the volume of intraoperative blood transfusion and postoperative complications, the prevention of perioperative bleeding has long been considered an important goal to reduce adverse outcomes.[1]

Usually the amount of blood used is determined by the amount lost during surgery, preoperative haematocrit and transfusion triggers used by anaesthesists; however, the current practices of liver recipient management have led to a steady reduction in transfusion requirements. In recent years, an increased number of patients undergo surgery without the need for any blood product transfusion.[2]

Improved results have been obtained through a better understanding of the haemostatic disorders associated with end-stage cirrhosis, improvements in organ preservation, surgical technique, and intraoperative anaesthesiological care. Continuous point-of-care monitoring of haemostasis associated with new pharmacological strategies adopted to correct haemostatic abnormalities have also significantly contributed to individualise the patient's need of blood products and reduce their total consumption.

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Coagulation Abnormalities of End-stage Cirrhotic Patients: Clinical Evidence for a Re-balanced Haemostasis

The complex coagulopathy of end-stage liver disease results from a variety of quantitative and qualitative deficiencies of the pro- and anticoagulant plasma proteins. The coagulation system is adversely affected by low levels of prothrombotic factors and antithrombotic modulators at the same time. In fact if, on one hand, failure to synthesise factors II, VII, IX and X results in coagulopathy, identified by prolonged Prothrombin time and activated thromboplastin time, on the other hand, a low level of antithrombotic compounds may result in a hypercoagulable state.

Fibrinogen level is generally normal or increased, but excessive sialic acid content in fibrinogen molecules results in the synthesis of functionally abnormal fibrinogen.[3]

The fibrinolytic system is also affected by liver disease, with the presence of low levels of plasminogen and α2-antiplasmin, and increased levels of tissue plasminogen activator. A reduction in platelet count and platelet function is also present; however, high levels of the platelet adhesive protein von Willebrand factor, together with low levels of its regulator ADAMTS13, ensure conservation of normal platelet function, and platelet number reduction therefore does not result in major bleeding. In addition, an increased potential to generate thrombin has been observed, due to an increase in plasma levels of coagulation factor VIII coupled with a decrease in plasma levels of protein C.[4]

Rather than a consequence of impaired coagulation, most bleeding complications in patients with liver disease are specifically related to alterations in haemodynamics and vessel wall function, and in particular to the hyperdynamic circulation and the presence of portal hypertension.

Due to "rebalancing" of the haemostatic system, standard coagulation tests do not necessarily reflect *in vivo* haemostasis, and it is debatable whether correction of abnormal coagulation tests is really necessary prior to surgery.[5]

Traditional attempts to prophylactically reverse preoperative abnormal coagulation tests by the administration of fresh frozen plasma (FFP) or platelet concentrates can no longer be recommended. Moreover, transfusion of blood products is associated with substantial side-effects, including fluid overload.

Surgical Techniques to Reduce Blood Loss

The importance of surgical experience and skill during hepatic dissection, as well as the performance of scrupulous haemostasis have long been recognised to be of key importance in determining the amount of perioperative blood loss. As the intraoperative events proceed, the already substantial coagulopathy of these patients is further altered, requiring meticulous attention to control even minimal sources of bleeding. Many technical details such as careful mobilisation of the liver, segmental vascular clamping, bloodless dissection of the inferior vena cava, introduction of modern devices for liver dissection, prompt haemostasis of the small vascular structures, etc, can make the difference in reducing bleeding complications. Generally, blood loss is directly proportional to the duration of surgery, and long operating times very often translate into higher transfusion requirements.

The use of veno-venous bypass during the anhaepatic phase has been introduced to decompress the splanchnic and retroperitoneal circulations and thus avoid excessive bleeding from portal hypertension and splancnic organ congestion (classical technique). Preservation of the vena cava (piggy-back technique) has been instead advocated to improve physiological parameters of body temperature, cardiac output, tissue perfusion and acid-base status. Proponents of the piggy-back technique claim that it avoids extensive dissection of the retroperitoneum area, reduces the anhepatic time, shortens warm ischaemia time during implantation and reduces the requirement of blood products.[6]

Currently, however, there is no evidence to recommend either approach over the other, and the recent statements from the Cochrane database show no superiority of one over another technique in reducing blood loss.[7]

Anaesthetists's Impact on Bleeding Control During OLT

Serious vasodilation of end-stage liver disease, important reduction in venous return, complicated stages of the procedure and haemodynamic deterioration may require unpredictable fluid resuscitation. Generous administration of blood products has been adopted to normalise the patient's coagulopathy and restore adequate haemodynamics. However, administration of large volumes is neither beneficial nor supported by evidence, and may result in further exacerbation of coagulopathy. The dilution of coagulation factors and platelets may in fact lead to a paradoxical increase in transfusion requirements.

Anaesthesiological conduct, focused on properly managing the haemodynamic status and fluid balance, along with monitoring and correction of coagulation abnormalities, may play an important role in reducing perioperative bleeding.[8]

Strategies aiming at the reduction of central and portal venous blood pressure have been associated with a reduction in perioperative blood loss in cirrhotic patients undergoing liver surgery.[9] Even though during OLT there is no evidence to support decreasing CVP and effective circulating blood volume to levels currently practiced during hepatic resection surgery, many anaesthesists support this strategy.[10,11]

Intraoperative lowering of the CVP by fluid restriction, diuresis induction and avoidance of routine transfusion of blood products, may assist in bleeding control by decreasing the blood pressure gradient over which bleeding occurs. After reperfusion, a low central venous pressure creates a venous pressure gradient between the portal and central venous circulation that draws blood through the donor graft.

However, circulatory instability commonly ensues during surgery and places patients at risk of organ hypoperfusion, and in particular renal hypoperfusion. Because of the typical vasodilated hyperdynamic physiology of this patient group, a valuable management of a hypovolaemic cirrhotic patient may rely on the administration of vasopressors. Cautious use of vasoconstrictors, by increasing systemic vascular resistance, may improve blood pressure and organ perfusion while avoiding positive fluid balance and limiting splancnic blood flow. Norepinephrine and vasopressin are often the agents of choice because of their potential to improve circulatory stability and enhance renal perfusion without inducing mesenteric ischaemia.

Using intraoperative lower tidal volumes and avoiding high positive end-expiratory pressure may further help in minimising preload and may therefore decrease the risk for bleeding.

Lowering the transfusion threshold and lowering targeted haemoglobin (Hb) concentration are currently proposed by many anaesthesists; it appears that over time there has been convergence towards an "acceptable" target Hb of approximately 10g/dL even in the setting of liver transplantation surgery.[12,13]

However, if 10g/dL remains a reasonable transfusion target for the

more severely ill liver recipients, the possibility of further reducing RBC transfusion, without adversely affecting outcomes, could be achieved by targeting even lower Hb levels in selected individuals who are less ill.

Recent research has pointed out the validity of fibrinogen administration in order to increase the stability and firmness of the fibrin clot. In the presence of surgical bleeding, and in accordance with the results of FibTem test on rotation thromboelastometry (ROTEM), fibrinogen administration during OLT was associated with a diminished rate of red blood cell components, fresh frozen plasma and platelet concentrate transfusion per patient. In a recent study, the use of fibrinogen resulted in 20% of transplant patients receiving no transfusions of blood products at all, compared with 3.5% in the pre-ROTEM period.[14]

In the presence of functional or quantitative deficits of fibrinogen, the use of fibrinogen concentrates should be considered a first-line therapy to correct perioperative bleeding, and to allow the reduction in transfusion of allogeneic products on an empiric basis.[15]

By accepting a haematocrit of 25–30%, maintaining a low CVP (<5-8mmHg), being very restrictive in transfusing FFP and platelets, and utilising fibrinogen when necessary, a conservative transfusion policy may still guarantee a satisfactory oxygen delivery, while preventing hypervolaemia and important side-effects of blood and plasma product transfusions.

Timely Monitoring of Coagulopathy

Close monitoring of coagulation disorders and other biochemical parameters is strongly recommended to diagnose deficits of clotting products, ongoing fibrinolysis, and the presence of heparin-like effect. "In theatre" Thromboelastography (TEG) or ROTEM® are currently used in many institutions, and their utilisation permits the assessment of both cellular and humoral components of whole blood coagulation and fibrinolysis, instead of singular parameters of procoagulation or anticoagulation. Point-of-care devices allow for the immediate appropriate treatment of coagulation disorders, as they can detect functional alterations in the interaction between cellular elements and plasma factors that are missed by the standard coagulation tests.[16,17]

Several studies have shown the usefulness of point-of-care coagulation monitoring during OLT, making it possible to decrease both transfusion volumes and the number of patients undergoing transfusions.[18,19]

Antifibrinolytic Drugs to Minimise Blood Loss

Increased fibrinolytic activity is observed in various stages of surgery, but preferentially after reperfusion, and represents one of the major causes of excessive bleeding during OLT.

The most widely used antifibrinolytics include aprotinin in the past years and nowadays tranexamic acid and epsilon-aminocaproic acid (EACA). Aprotinin has been abandoned due to the increased associated risk of thromboembolic events in cardiac surgery and the potential of inducing renal dysfunction. However, in a large patient population, Warnaar *et al*,[20] did not demonstrate an increased risk of thrombotic complications or mortality when aprotinin was used during OLT.

In liver transplant surgery, the effectiveness of tranexamic acid in reducing blood transfusion requirements is still under critical evaluation. In fact, even though both high- and low-dose administration has significantly reduced the use of intraoperative blood products,[21,22] a recent report by the Cochrane Hepato-Biliary Group[23] demonstrated that there were no significant differences in the allogeneic blood requirements, amount of platelets, FFP, or cryoprecipitates transfused between the tranexamic acid and control groups. Variable rates of success have been demonstrated in EACA's ability to control significant bleeding, but the optimal dose has yet to be firmly determined. The routine use of antifibrinolytics has been debated because of concerns about hepatic artery thrombosis and other thromboembolic events. It is not recommended to administer these agents in a prophylactic manner, but only if fibrinolysis is demonstrated by thromboelastography, and preferably after an adequate intra-hepatic blood flow re-establishment. A recent critical meta-analysis did not show a higher incidence of these feared complications with the "liberal" use of antifibrinolytics.[24]

Recombinant Factor VIIa

Even though it increases the thrombin burst and acutely improves coagulation in the presence of rapid factor consumption, it has not yet been demonstrated that recombinant Factor VIIa reduces blood product transfusion. Therefore, there is no clear evidence to promote the use of rFVIIa in liver transplantation surgery. In a recent meta-analysis there were no significant differences in blood transfusion requirements between patients who received rFVIIa and those who received a placebo.[25]

Cell Salvage Autotransfusion

Although it can be considered a safe and valuable alternative to reduce allogeneic red blood cell transfusions, the routine use of cell saver devices is still controversial during OLT. However, cell salvage is cost-effective and reduces some complications of homologous transfusions, including citrated products, transmission of infections, metabolic derangements, and coagulopathy. Collection and reinfusion of autologous blood results in conservation of erythrocytes, substantial reduction in FFP and a reduction in platelet requirement.[26]

Unsolved concerns on the routine use of blood salvaging techniques include its cost-effectiveness and the suggested increased risk of

coagulopathy development. It has been hypothesized, in fact, that the release of some compounds from blood cells in the collected blood might induce fibrinolysis, with a subsequent paradoxical increase in transfusion requirements.[27]

In conclusion, haemostatic alterations of patients with end-stage liver disease may not be as clinically relevant in determining intraoperative blood loss as traditionally assumed.

Although OLT is often associated with haemorrhage, the prophylactic correction of clotting defects in the absence of clinical bleeding is no longer recommended, and a substantial proportion of patients can nowadays be transplanted without the requirement of any blood product transfusion. Optimal management of coagulation, which also includes the point-of-care guided use of fibrinogen and antifibrinolytic agents, is able to reduce significant surgical haemorrhage, prevent large amount of allogeneic blood product transfusion, and is cost-effective. Over and above the conservation of resources, a decrease in transfusion provides crucial patient benefits in terms of perioperative morbidity, and minimises one of the most important risk factors for mortality after OLT.[28]

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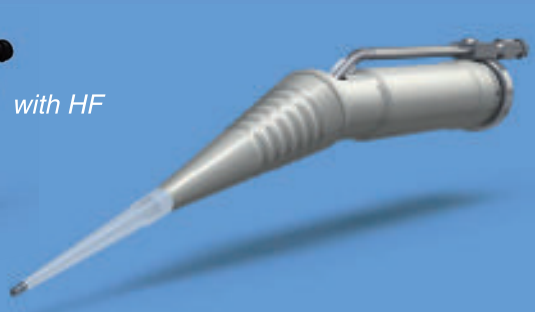

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Towards an Integrated Management Strategy for Solitary and Polycystic Disease of the Liver

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Benign, non-parasitic, cystic disease of the liver is most frequently encountered in the form of solitary simple cysts, often as an incidental finding on abdominal imaging.[1] Computed tomography-based estimates suggest that 18% of the population have simple hepatic cysts[2] and due to slow growth and small size they are rarely symptomatic. Polycystic disease affecting the liver reflects a phenotype resulting from two distinct inherited conditions. Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited nephropathy affecting up to 0.2% of the population,[3] whereas autosomal dominant polycystic liver disease (PCLD) has a prevalence of less than 0.01%.[4] The last decade has witnessed significant advances in the understanding of the pathogenesis and the management of polycystic disease with recent evidence in support of disease modifying agents and in interventional radiological techniques.[1] However, the optimal approach of this complex disease remains elusive due to the paucity of experience in individual centres. Centralisation of specialist services and multicentre collaborative trials offer the opportunity for a more robust evaluation of the safety and effectiveness of individual treatment options. These are likely to be individually tailored to size, location and volume of cysts, patient fitness and the burden of morbidity and mortality associated with each option. Here we review recent advances in our understanding of simple and polycystic disease of the liver and propose an integrated management strategy.

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Pathogenesis

ADPKD is associated with mutations in PKD1 gene expressing polycystin-1, accounting for 85% of mutations in autosomal dominant families, and PKD-2 mutation expressing polycystin-2.[5] Hepatic cysts are the commonest extra-renal manifestation of the disease with PKD1 mutation characterised by an aggressive form of the disease, with early onset of symptoms and 67% to 83% of patients having progressive cystic involvement of the liver with advancing age.[6] The other inherited condition is the less prevalent isolated polycystic liver disease (PCLD) and hepatic cystogenesis is associated with germ-line mutations of genes encoding hepatocystin (PRKCSH),[7] SEC 63[8] (encoding SEC63p) and low density lipoprotein receptor related protein 5 (LRP5).[9] The formation of liver cysts is the result of congenital malformation of the hepatic ductal plate and cilia of cholangiocytes.[10] Insufficient resorption of the ductal plate leads to large dilated segments of the primitive bile duct, which causes cyst formation (Figure 1). The resulting abnormal biliary ductules are not connected to the intrahepatic biliary system and undergo progressive enlargement. This causes compression of the adjacent liver tissue, distorting the organ architecture, localised atrophy and enlargement of contralateral segments but with generally preserved organ function.

Clinical Presentation

The majority of patients with polycystic liver disease will remain asymptomatic.[6,11] With an annual growth rate of 0.9 to 3.2%,[6] symptom-based presentation occurs at a mean age of 50 years, with predominance in women (79.4% to 94.7%). Although the natural history of growth and the genetic basis dictate equal gender prevalence, the higher incidence in females suggests stimulating effects of female steroid hormones, as

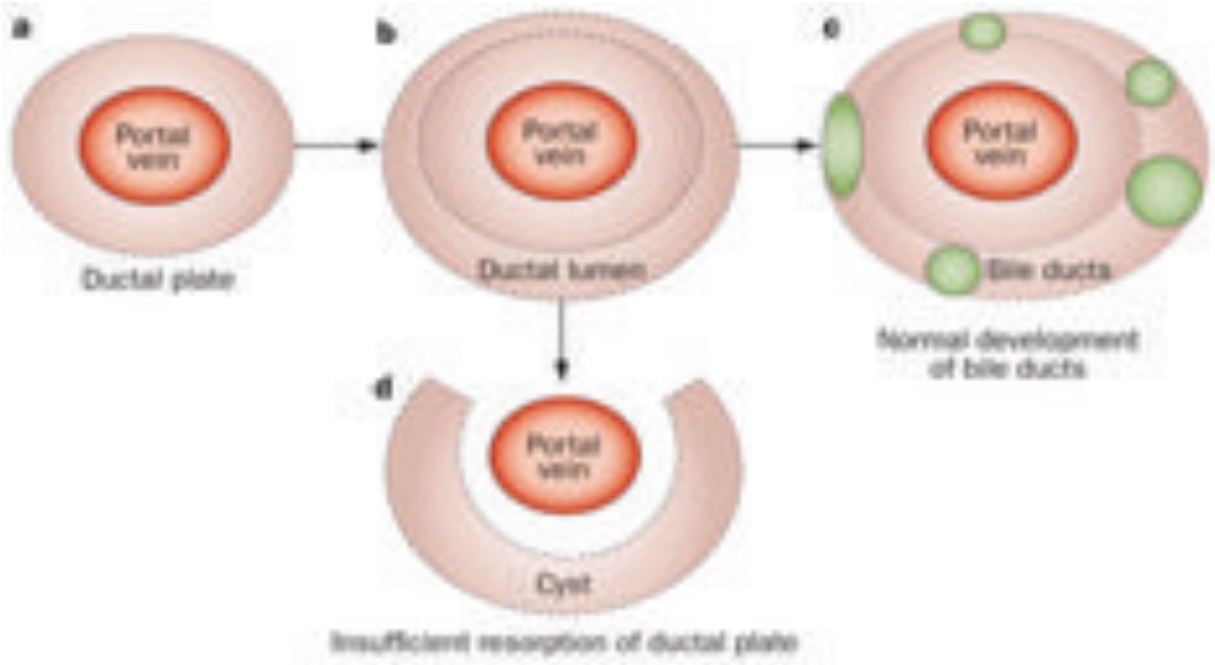

Figure 1. Embryonic development of the ductal plate. a. During early embryogenesis, a single-layer ductal plate surrounds the portal vein. b. Double-layered plates are then formed. c. Resorption of the ductal plate leads to the formation of a network of bile ducts. d. Insufficient resorption of the ductal plate leads to large dilated segments of the primitive bile duct and is considered to be the cause of cyst formation. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Gastroenterology & Hepatology, Gevers *et al.*,[1] copyright 2013.

use of exogenous hormones and multiple pregnancies are associated with increased growth. This stimulatory effect on growth seems to affect hepatic but not renal cysts.[12]

Symptoms are produced by the large liver volume causing post-prandial fullness, dyspnoea and upper abdominal pain or result from complications such as bleeding, infection, torsion, intrahepatic venous thrombosis or rupture. Rarer complications include portal hypertension and gastrointestinal bleeding, biliary obstruction or inferior *vena cava* obstruction.[13] Despite significant liver volume enlargement and distortion of the parenchyma, liver function profile remains normal in the majority of patients with changes usually only seen in alkaline phosphatase and gamma glutammyl transferase levels, which are elevated in 70% and 27% of patients respectively.[11] Extra-hepatic manifestations of polycystic liver disease include intracranial aneurysms (6-16%) and mitral valve prolapse (25%). Further research is required before routine screening for these conditions can be recommended.[14]

Management of cystic disease of the liver relies on accurate diagnosis as other pathologies must be excluded. For solitary cysts, hydatid disease with negative serology must be considered as an alternative diagnosis, along with intracystic haemorrhage, biliary cystadenoma or cystadenocarcinoma.[15] Polycystic liver disease is associated with an adverse impact on quality of life and is associated with poor general health, functional status and social functioning.[16] Treatment of solitary or polycystic liver disease is only indicated in symptomatic patients due to its benign, albeit progressive, nature. The aim of treatment is to reduce the overall liver volume or reduction of strategic cyst size to alleviate symptoms.

Non-surgical Interventions

Although surgical management has been the mainstay of treatment for symptomatic polycystic liver disease, effective alternatives have developed in the last decade for patients who may be unfit or not suitable for surgical intervention. Disease modifying agents such as somatostatin analogues and mammalian target of rapamycin (mTOR) inhibitors and transarterial embolisation of the dominant cysts are emerging treatment options that provide alternatives and adjuncts to the existing management strategies.[1]

Aspiration sclerotherapy, in the form of ultra-sound guided aspiration and chemical ablation of the secretory epithelium is most suited to single (>5cm diameter) or dominant cystic disease. It can be undertaken in an outpatient setting. Ethanol is the most commonly used sclerosant and the basis of therapy is the destructive effect on the cyst epithelium to prevent recurrence. Minocycline and Tetracyclines have also been used due to the destructive effect on the epithelium caused by their low pH. A review of 34 articles incorporating 292 patients reported a complete regression in 22% with a partial response in a further 19% of patients.[1,6] Recurrence was reported in 21% of patients in the follow-up period but most of them remained asymptomatic or reported significant improvement. Although the procedure is associated with minimal morbidity, the benefit of significant volume reduction of 17% to 19% is offset by the need for multiple procedures to maintain therapeutic effect.[1,17]

Transarterial embolisation (TAE) has emerged as a viable treatment

option in the last decade.[1,18,19] Early experience suggests that the procedure may be difficult due to alteration of the intrahepatic vascular structure, but it is associated with a significant reduction of the cysts' size and increase in normal liver parenchyma.[18] A recent retrospective review of 244 symptomatic ADPKD patients with polycystic liver disease undergoing hepatic TAE demonstrated a progressive decrease in liver volume by 9.2% by one year without significant morbidity.[19] It is a useful addition in the armamentarium against symptomatic disease in patients in whom surgical intervention may be contraindicated.

Disease Modulating Therapy

The influence of female steroid hormones on polycystic disease of the liver has identified a modifiable risk in the management of progressive disease and patients should be discontinued from ongoing hormone therapy.[1] Modification of the natural history of the disease can be achieved by reducing cyst fluid secretion and proliferative changes in cholangiocytes. Somatostatin inhibits intracellular 3'-5' cyclic adenosine monophosphate (cAMP), an important 2nd messenger for cholangiocyte proliferation and cyst fluid secretion. The first randomised controlled trial evaluating the long-acting somatostatin analogue, lanreotide in 54 polycystic liver disease patients, demonstrated good tolerability with reduction in both renal and hepatic cystic disease.[20] However, the reduction was noted to occur mostly in the first 6 months of the study with return to increasing cyst size after cessation of treatment.[1] A second trial evaluating long-acting octreotide in 42 patients over one year reported a reduction in liver cyst volume of 4.9% compared to an increase of 0.9% seen in the placebo group.[21] This modest reduction implies sustained control of a progressive disease but may offer limited symptomatic response.[20] Furthermore, a recent pooled analysis of 107 patients from three randomised placebo-controlled trials found somatostatin analogues to be more effective in cyst reduction for women younger than 48 years with 6 to 12 months of therapy.[22] These attributes, along with the high cost of treatment, may limit its application as an adjunct to surgery or as the only therapy for patients who may not be candidates of surgery or liver transplantation.

mTOR plays a role in cystogenesis and its inhibition has an antiproliferative effect on cholangiocytes. In a trial of 16 patients with ADPKD-associated renal failure and transplant, the mTOR inhibitor sirolimus demonstrated a 16% reduction of liver and renal cysts compared to an increase in cyst size of 14.2% with other immunosuppressive agents.[23] However, a subsequent randomised controlled trial evaluating combined everolimus and octreotide therapy, found a minimal non-significant additional reduction in cyst size compared to octreotide alone (3.5 vs. 3.8%).[24] Although the added role of mTOR inhibitors continues to be evaluated, they are likely to be a palliative option in conjunction with somatostatin analogues in patients who are not candidates for surgery.

Further research is clearly warranted to ascertain the durability of medical management, its long term safety, its cost effectiveness as well as clinical investigation of emerging novel somatostatin analogues.

Surgical Interventions

Due to the recurrent and progressive nature of the disease, surgery has been the mainstay of management. Although the classification of polycystic liver disease has been arbitrary at greater than 20 cysts, clinically useful diagnostic tools have been proposed to guide surgical intervention. Gigot *et al.*[25] proposed a classification to guide surgical fenestration (Figure 2), with type I disease representing fewer than 10 cysts but greater than 10cm in diameter. Surgical fenestration is advocated for this form of disease. Type II represents a diffuse disease with dominant cysts and significant sparing of liver, where hepatic resection can be considered in addition to fenestration. Type III represents the most severe form of the disease with diffuse liver involvement with small and medium sized cysts and minimal parenchymal sparing. For this type, early consideration of liver transplant is proposed. Other classifications have been proposed, tailored to monitor progress in the context of familial screening[26] or to

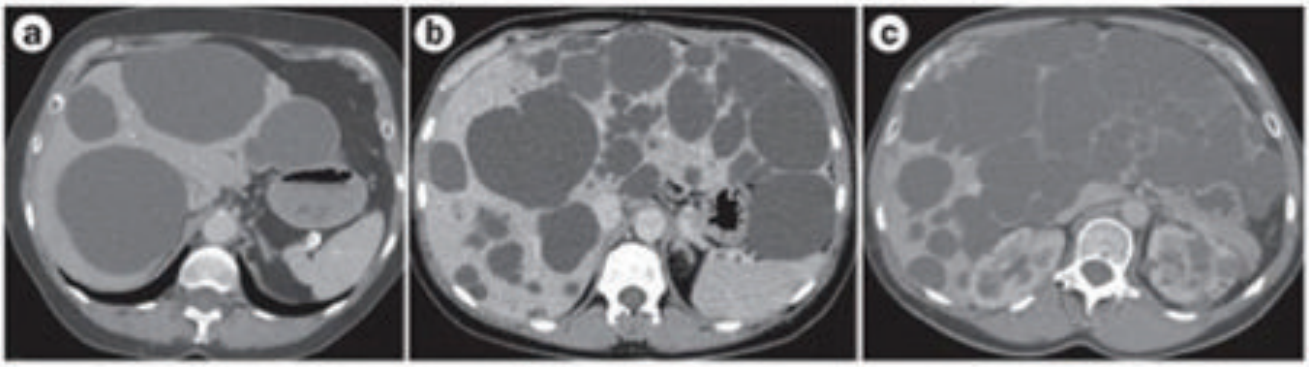

Figure 2. Transverse CT images of polycystic liver disease. a. Gigot type I cystic liver containing a couple of large (>10cm) cysts, but <10 cysts in total. b. Gigot type II polycystic liver with diffuse involvement of liver parenchyma by multiple medium-sized cysts. c. Gigot type III polycystic liver. The liver is completely occupied with numerous cysts, and only a few areas of visible liver parenchyma are present. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Gastroenterology & Hepatology, Gevers *et al.*,[1] copyright 2013.

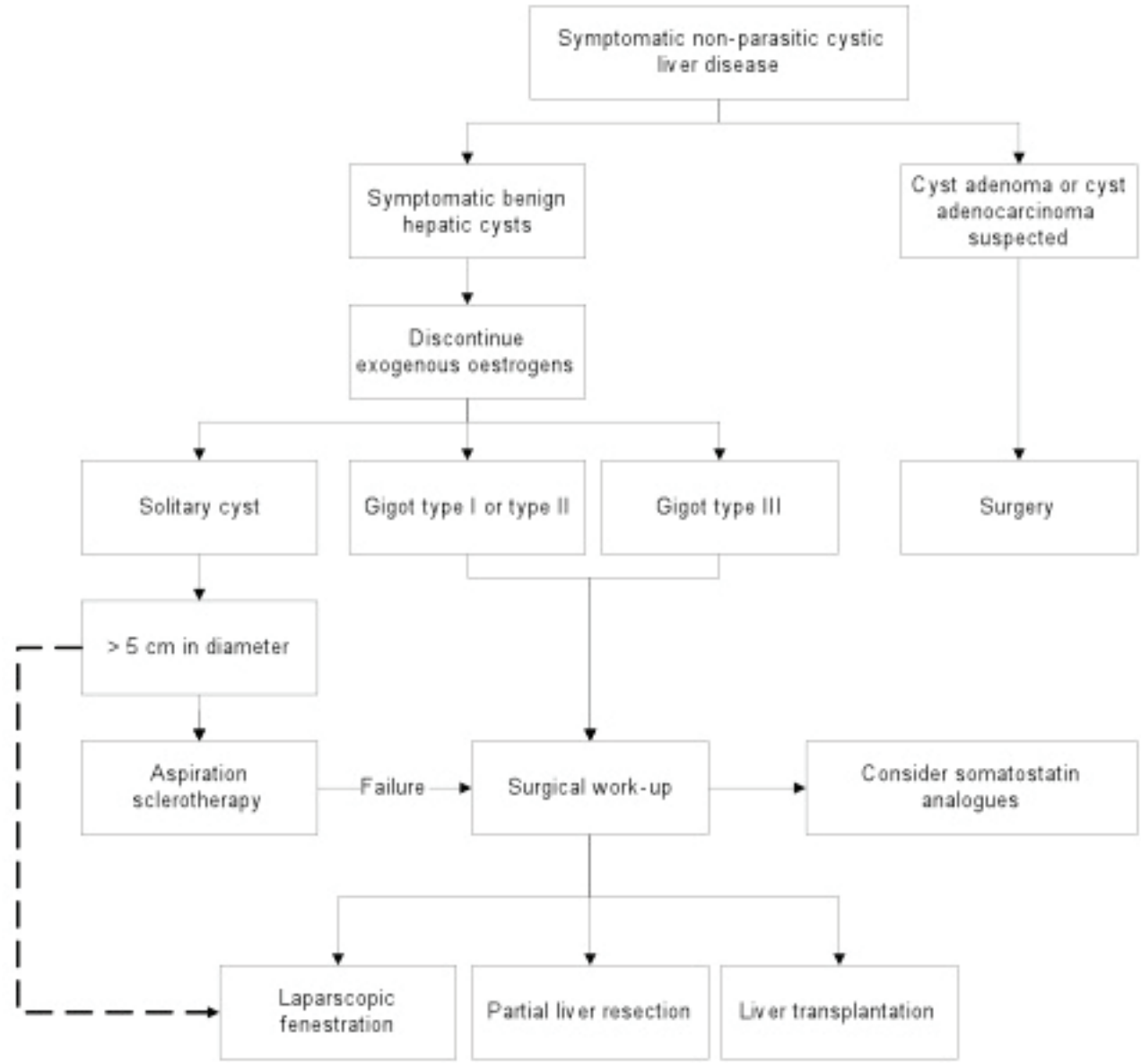

Figure 3. Management pathway for symptomatic non-parasitic benign cystic liver disease.

establish the risk and benefits of liver transplantation.[27]

Fenestration (or de-roofing) combines aspiration with surgical de-roofing of the cysts. Coagulative ablation is aimed at destroying the remaining cyst epithelium. It is best suited for solitary or Gigot type I and II disease. Surgical fenestration allows access to multiple cysts at the same setting, offering greater symptomatic relief and liver size reduction than aspiration-sclerotherapy alone. A review of the 43 articles reporting outcomes of surgical fenestration in 311 patients reported an increasing trend towards a laparoscopic approach.[6] Eighty per cent of all procedures were undertaken laparoscopically, with an open conversion rate of 22%. Symptomatic relief was reported in 92% of patients with cyst recurrence in 24% and symptom recurrence in 22% of patients in the follow-up period. Mortality from both approaches was 2%; the laparoscopic approach is the treatment of choice as it offers lower perioperative morbidity (29% vs. 40%).

Laparoscopic fenestration may be difficult with deep seated cysts, location of cysts over the dome of the liver (segments 7 and 8) and because of adhesions from previous surgery. This can result in ineffective outcomes due to incomplete fenestration of cysts.[6]

Hepatic resection (with or without fenestration) may be considered in Gigot type II disease due to the often asymmetrical distribution of cystic disease. Open surgery and resection may be the primary intervention in cases of diagnostic uncertainty or massive incapacitating hepatomegaly.[6] It can offer adequate volume reduction and allow liver regeneration in the space created by removal of the cystic segments. Surgical resection can however be challenging due to the significant enlargement and rigidity of the liver, which can make access to the hepatic veins difficult.[6, 27] Furthermore, the altered intra-hepatic vasculature, collateral circulation and altered biliary anatomy can result in post hepatectomy haemorrhage and bile leaks.

Due to the small number of patients undergoing hepatic resection for polycystic liver disease, most large centres have limited experience and report variable outcomes.[6] A review of the literature found 26 articles, totalling 337 patients undergoing resection for polycystic liver disease.[1] Perioperative morbidity was noted to be 51%, which was higher in patients with concomitant renal impairment and on immunosuppressive therapy after renal transplantation. Mortality was in excess of 3%. A direct correlation was noted for improved survival in high volume centres. Symptom relief was noted in 82% of patients with cyst recurrence of 34% in the follow-up period. The high recurrence rate and return of symptoms should warrant consideration of liver transplant early without hepatic resection in patients with incapacitating symptoms and poor quality of life as liver transplant surgery can be more challenging in the presence of adhesions.

Liver transplantation offers the only curative treatment for patients with Gigot type III or severe polycystic liver disease. As most patients continue to demonstrate normal liver function, liver transplantation in patients with severe, debilitating polycystic liver disease should be considered because of poor performance status and quality of life6 before the onset of malnutrition, which could lead to increased perioperative morbidity and mortality. A recent review of 29 articles reporting post-liver-transplant outcomes for polycystic liver disease in 206 patients found a perioperative morbidity of 41% and 30-day mortality of 3%. Three per cent of patients required retransplantation in the follow-up period. One- and five-year survival with liver transplant alone was noted to be 93% and 92% respectively, whereas, survival with combined kidney and liver transplant was poorer at 86% and 80% at these same time points. Although polycystic liver disease does not reduce life expectancy, liver transplant offers a significant improvement in quality of life for most patients. This benefit must be weighed against the risks of surgery and the long-term risks of immunosuppressive therapy.

Towards an Integrated Management Strategy for Simple and Polycystic Disease of the Liver

Polycystic disease of the liver is a complex disease with many treatment options. Symptomatic disease or complicated cysts warrant treatment. The optimal approach depends on the severity of symptoms and the presence of complications of the disease. Although there is no consensus on the optimal management algorithm, many pathways have been proposed that highlight a pragmatic and tailored approach to individual patients, taking into account cyst size, location, possibility of malignancy, medical co-morbidities and technical feasibility.[25, 28, 29] The mainstay of management in early or moderate disease relies on minimally invasive techniques of aspiration sclerotherapy and laparoscopic fenestration. Open surgery combining resection with fenestration will continue to play a role, especially in cases of diagnostic uncertainty. Transarterial embolisation and disease modulating therapies in the form of Somatostatin analogues and mTOR inhibitors can provide an adjunct role to other therapies or offer an effective tool in the control of symptoms and disease progression in patients unfit for surgery and deserve further investigation. Liver transplantation remains the only curative option for those with advanced disease and poor quality of life (Figure 3).

Conflicts of interest:

The authors state that there are no conflicts of interest.

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Upcoming Congresses and Meetings

2014 Joint International Congress of ILTS, ELITA & LICAGE

4 - 7 June 2014

London, UK

The 2014 Joint International Congress of ILTS, ELITA & LICAGE brings together over 1200 professionals from all over the world, dedicated to promoting and learning about the scientific advances in liver transplantation.

The congress gives attendees the opportunity to interact, network, and discuss the latest advances in liver transplantation procedures and therapy.

http://2014.ilts.org

47th Annual Meeting of The European Society for Paediatric Gastroenterology, Hepatology and Nutrition

9 - 12 June 2014

Jerusalem, Israel

The 47th Annual Meeting of ESPGHAN is taking place from Monday 9 to Thursday 12 June 2014, in Jerusalem, Israel. With an easy access venue located in the heart of the city's business and cultural centre, attendees will have easy access to the venue with, domestic and international transportation networks.

The meeting will focus on the recent advances in genetic, immunological, microbiological and clinical developments in the field of gastrointestinal disorders, liver diseases, and nutrition. It will provide attendees with many opportunities to discuss and share clinical and research field interests, present original work network with new colleagues.

www.espghan2014.org/home

European Society of Gastrointestinal and Abdominal Radiology (ESGAR) 2014

18 – 21 June 2014

Salzburg, Austria

A not-for-profit, educational and scientific organization, ESGAR was founded by radiologists working in close association with gastroenterologists, hepatologists, abdominal surgeons, pathologists, and other allied specialists. This year the 25th Annual ESGAR Meeting will take place in the City of Salzburg, Austria.

The programme will take the course of the established and appreciated ESGAR meeting format to include 90-minute lecture sessions, joint sessions with other societies and organisations.

www.esgar.org/annual-meeting/esgar-2014/

16th World Congress on Gastrointestinal Cancer

25 – 28 June 2014

Barcelona, Spain

The ESMO 16th World Congress on Gastrointestinal Cancer 2014 will be held during June 25-28, 2014 in Barcelona, Spain.

The ESMO 16th World Congress on Gastrointestinal Cancer 2014 will provide an update on the clinical updates in the management of patients, focusing on the care of patients with gastrointestinal cancer, including diagnosis and screening for common and uncommon tumors.

http://worldgicancer.com/wcgi/WGIC2014/index.asp

43rd Annual International Society of Hematology Meeting ILCA 2014 - The International Liver Cancer Association's 8th Annual Conference

5 – 7 September 2014

Kyoto, Japan

Back in Asia for the second time the ILCA brings the 8th Annual Conference from Kyoto, Japan.

The conference aims to advance liver cancer science and the care for liver patients worldwide by encouraging and stimulating scientific debate and networking between liver cancer professionals from all over the world.

The multidisciplinary scientific programme will include; State-of-the-Art Lectures; Symposia focusing on the advancements, research and treatment levels; General Sessions and e-Poster Viewing Tours; Luncheon Workshops; Industry Exhibition; Networking Breaks and Reception; and Consensus Workshop on Clinical Trial Design.

www.ilca2014.org/

2nd World Congress on Controversies in Gastroenterology (CIGI)

12 - 14 September 2014

Xian, China

Following on from the success of the 1st World Congress on Controversies in Gastroenterology, the 2nd World Congress on Controversies in Gastroenterology (CIGI) that will take place in Xian, China in September 12-14, 2014.

With overviews, state of the art lectures and controversial debates shaping the Scientific Program, a significant allocation of time for interactive debates and questions from the audience to each panel of experts.

The congress will provide attendees with the latest information along with opportunities to listen to key opinion leaders discuss controversial issues in the field of gastroenterology and endoscopy. Attendees are encouraged to take an active role in discussions following each debate.

www.comtecmed.com/cigi/2014/

The Cardiovascular and Interventional Radiological Society of Europe (CIRSE)

13 – 17 September 2014

Glasgow, UK

The CIRSE returns to the UK this year in Glasgow, which is easy to reach thanks to its three local airports and public transport.

CIRSE, is a non-profit association which focuses on improving patient care by encouraging innovations in the teaching of, research and clinical practice in the field of cardiovascular and interventional radiology.

The Scientific Programme has been refined this year and will various tracks for attendees to partake in. The event will encompass over 50 hours of vascular education which will include 15 Special Sessions, 12 Workshops, 4 Fundamental Courses and more than 10 Hands-on Workshops.

Key experts will debate the most contentious issues in the Controversies in SFA treatment and Controversies in BTK treatment sessions. Attendees also have other learning opportunities available to them, including an Interactive Case Session entitled Challenging venous interventions and a Hot Topic Symposium the Vascular Track offers ample learning opportunities for novice and expert alike.

www.cirse.org/index.php?pid=938

EASL Special Conference "Optimal Management of Hepatitis B Virus Infection"

25 - 27 September 2014

Athens, Greece

Organised by the European Association for the Study of the Liver (EASL) and the Asian Pacific Association for the Study of the Liver (APASL), this EASL Special Conference "Optimal Management of Hepatitis B Virus Infection" will be held in Athens.

The meeting aims to improve patient management and to identify and highlight medical needs and direct preclinical and clinical research in this field. With attendees from all over the world the meeting has been designed to generate discussions and interactions between clinicians and scientists to show the similarities and differences between different patient populations.

There will be a focus on the clinical aspects of the management of chronic HBV infection, as well as covering other topics such as HBV virology and immunology, HBV epidemiology, immunopathogenesis, natural history, treatment indications and end-points, optimal current treatment approaches and their challenges in chronic hepatitis B patients, including special groups, and finally innovative prospective treatment options.

https://events.easl.eu/EventPortal/Information/SPA2014/HOMEV2.aspx

Viral Hepatitis Congress 2014

9 – 11 October 2014

Frankfurt, Germany

This year the 3rd Viral Hepatitis Congress will take place from 9–11 October 2014 in Frankfurt, Germany. The organisers will present a high quality scientific programme made up of innovative and interactive discussion sessions.

With many new drugs treatment options coming to the market, a meeting such as this provides a vital forum for the wealth of data to be presented. The congress will collate and analyse the latest data from a broad range of sources, examine the new treatment options, and communicate this to the attendees.

The scientific programme will include innovative and interactive discussion sessions and will explore the clinical challenges presented by viral hepatitis infection and its associated complications.

www.viral-hep.org

1st International Congress on Biliary Atresia and Related Diseases (BARD)

16 – 17 October 2014

Berlin, Germany

The 1st International Congress on BARD will include up-to-date and innovative lectures from key opinion leaders from all over the world. The Scientific Program will be multidisciplinary to appeal to clinical researchers; pediatric and adult hepatologists and gastroenterologists; and pediatric hepatobiliary and transplant surgeons. With ample content covering biliary atresia, neonatal cholestasis and how to care for biliary atresia patients.

The congress will also host the launch of a new internet-based BARD registry.

http://www.bard-berlin-2014.com

CHRONIC LIVER DISEASE

A SLOW, SILENT, BUT PREVENTABLE DEATH

According to the World Health Organization, in 2008, 47.147 Europeans died of liver cancer, and 84.697 Europeans died of liver cirrhosis. These figures combined exceeded breast cancer mortality figures (103,255)[1].

Worldwide, both cirrhosis and liver cancer are on the rise.

THE CAUSES

The major causes of chronic liver disease are:

- Lifestyle (alcohol and overweight/obesity, leading to alcohol- or non-alcohol related fatty liver disease)
- Virus infection (mainly viral hepatitis B and C), and
- Genetic factors including autoimmune diseases

In addition, alcohol consumption by those who are infected with chronic hepatitis B and/or C multiplies the risk of developing cirrhosis and primary liver cancer[2].

ON THE POSITIVE NOTE, THE VAST MAJORITY OF CHRONIC LIVER DISEASE CASES CAN BE PREVENTED AND/OR TREATED

With the recent publication of its manifesto[3], ELPA calls on policymakers to ensure that an integrated approach, from prevention, to early diagnosis of a possible liver problem (e.g. via enzyme testing), to treatment, is taken to deal with the growing burden of liver disease.

ABOUT ELPA:

ELPA emerged from a desire among European liver patient groups to share their experiences. In June 2004, 13 patient groups from 10 European and Mediterranean Basin countries met to create the association. ELPA which formally launched in Paris, on April 14th 2005, now has 29 members from 24 countries.

ELPA and its members are dedicated to multi-level initiatives involving EU and national policymakers, liver specialist associations and public health experts.

European Liver Patients Association

ELPA

For more information about ELPA, please visit our website at **www.elpa-info.org** or contact:

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